

Gamma Irradiation of Hydrolyzed
Heart Valve Cusps in the Presence of PPG 400

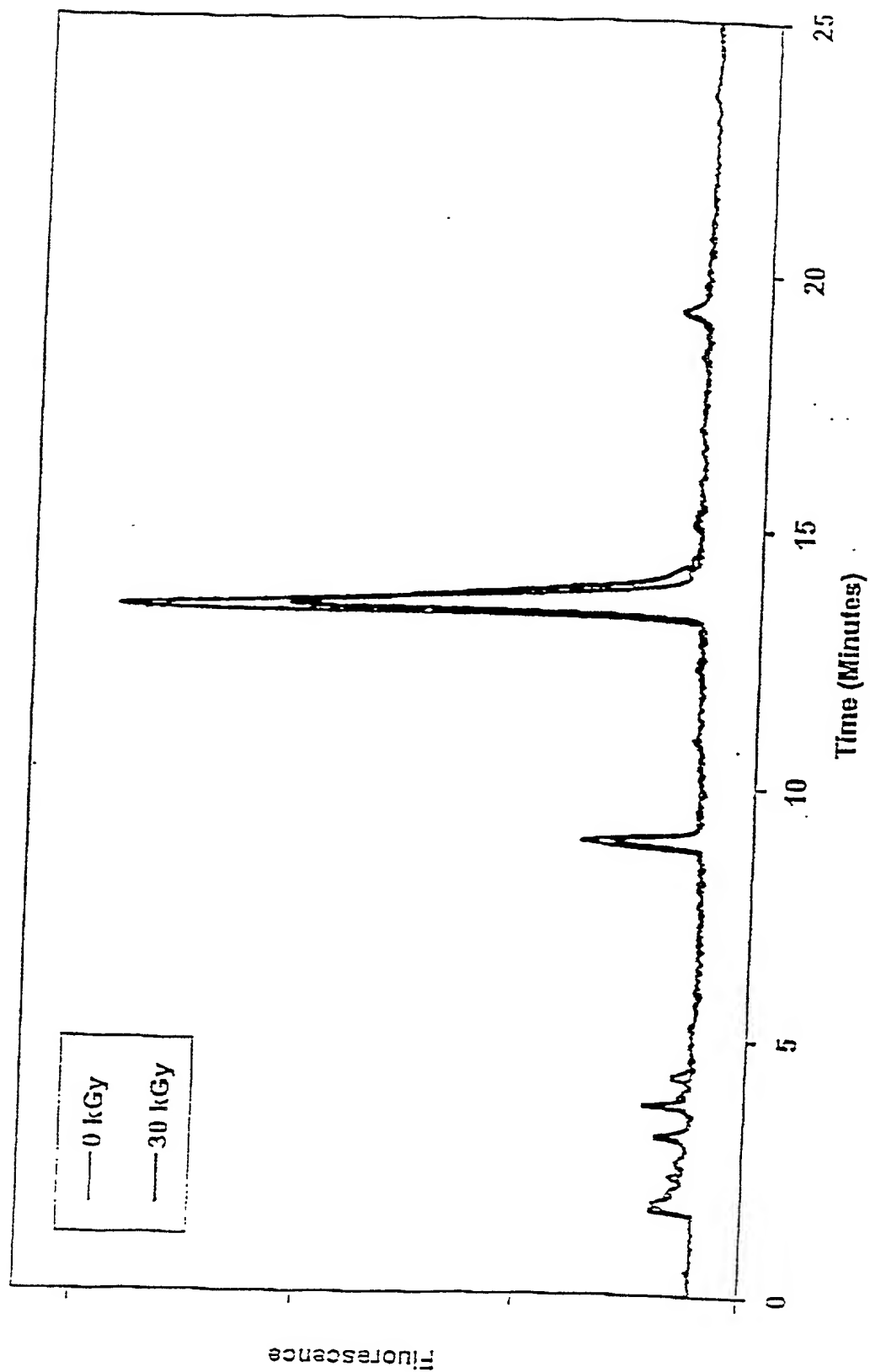


Figure 1a

Gamma Irradiation of Hydrolyzed Heart Valve
Cusps in the Presence of PPG 400 and 125 mM Trolox C

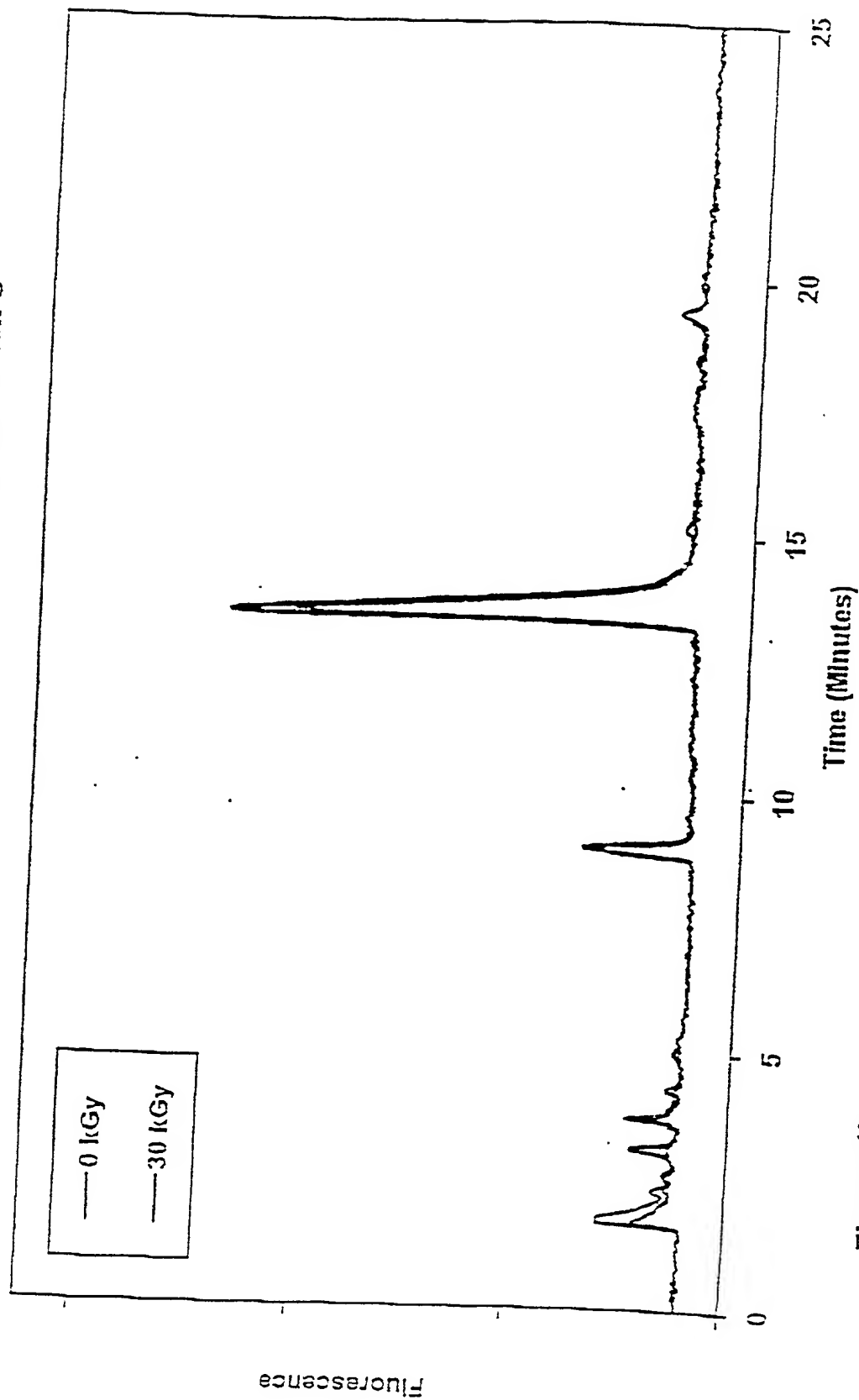


Figure 1b

Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the Presence
of PPG 400 and a Stabilizer Mixture of 62.5mM Trolox, 100mM Lipoic
Acid, 100mM Coumaric Acid, and 100mM n-Propyl Gallate

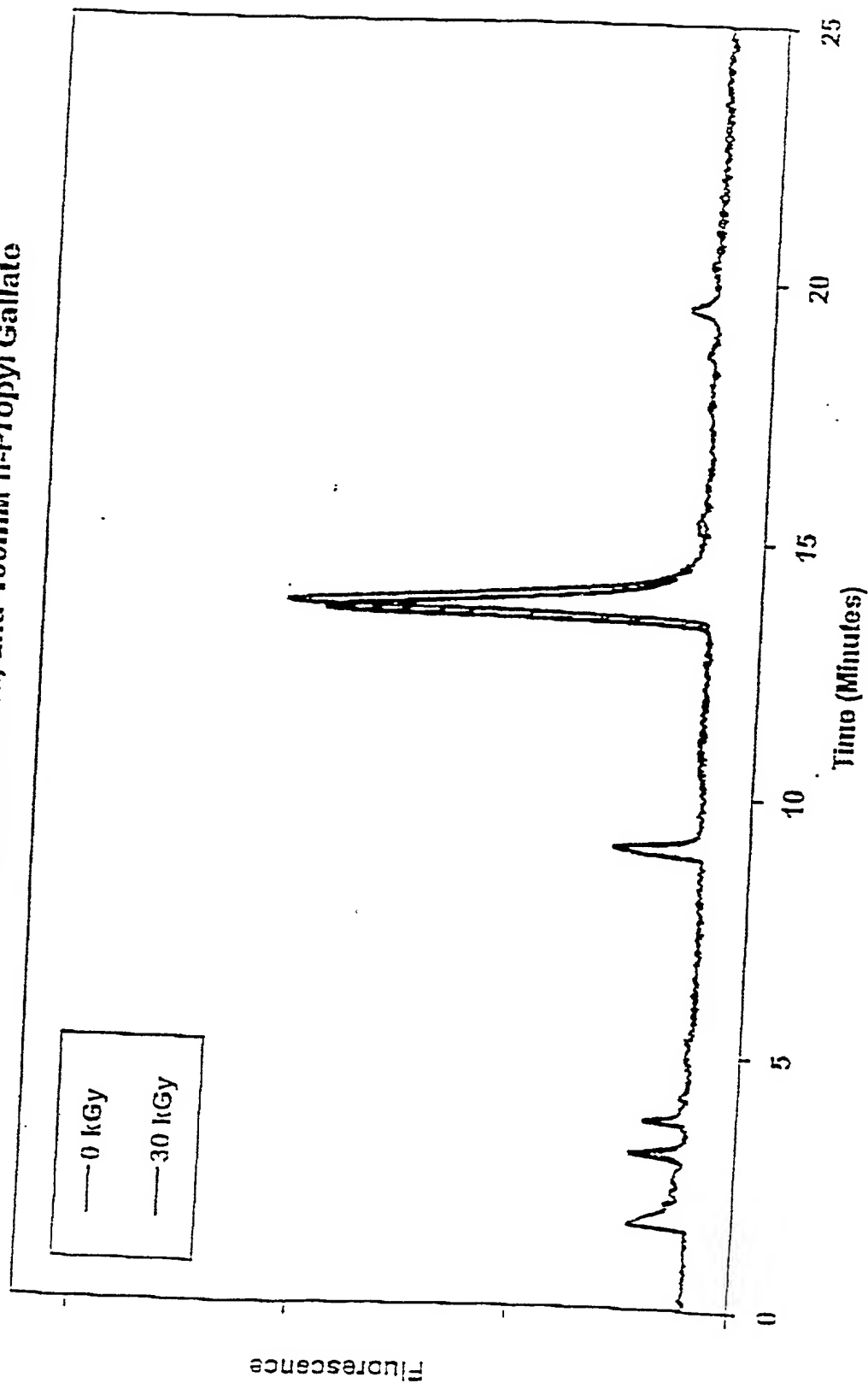


Figure 1c

Valve Cusps in the Presence of PPG400 with Various Stabilizers

Gamma Irradiation of Porcine Heart

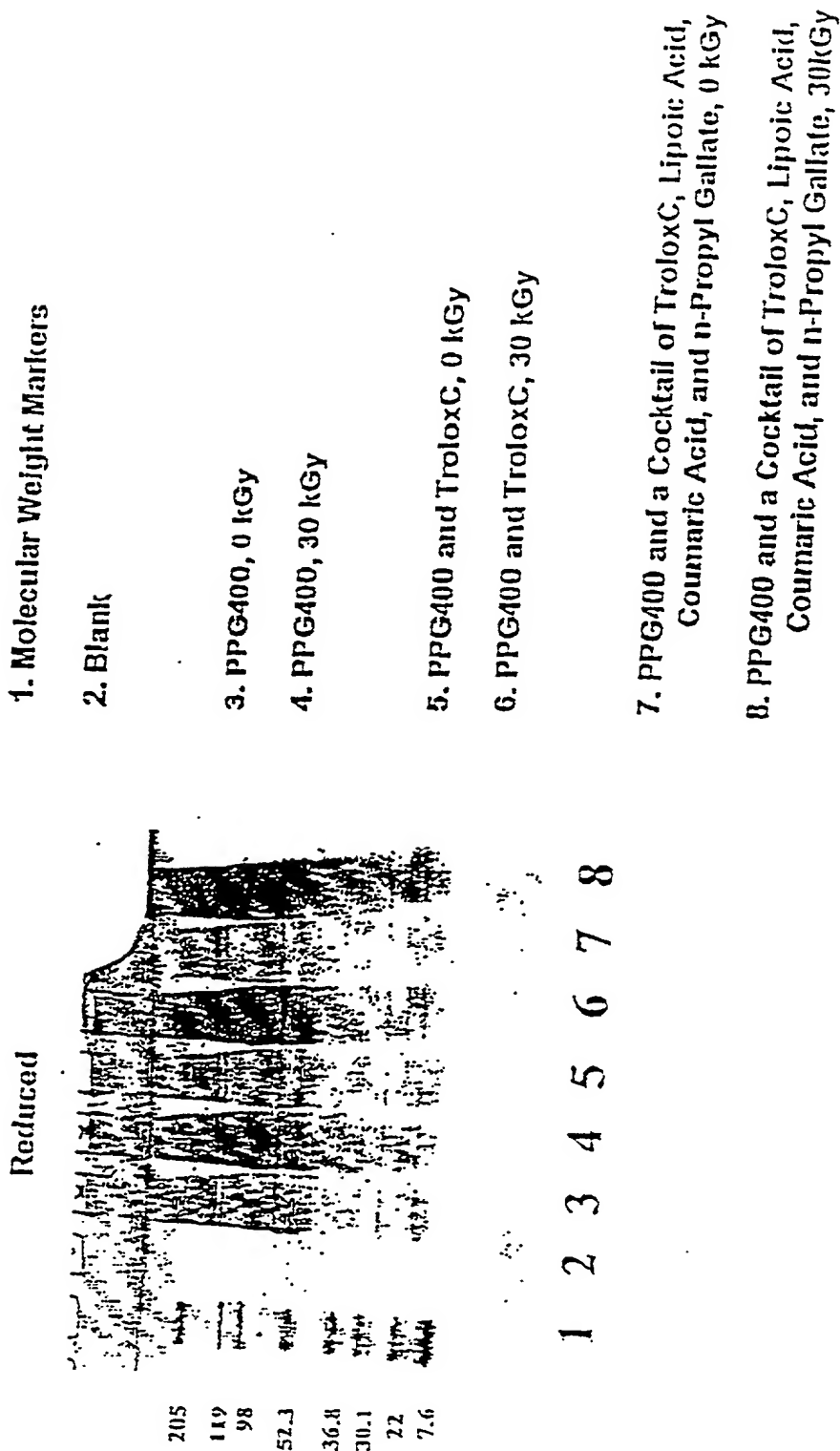


Figure 1d

Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the Presence of PBS

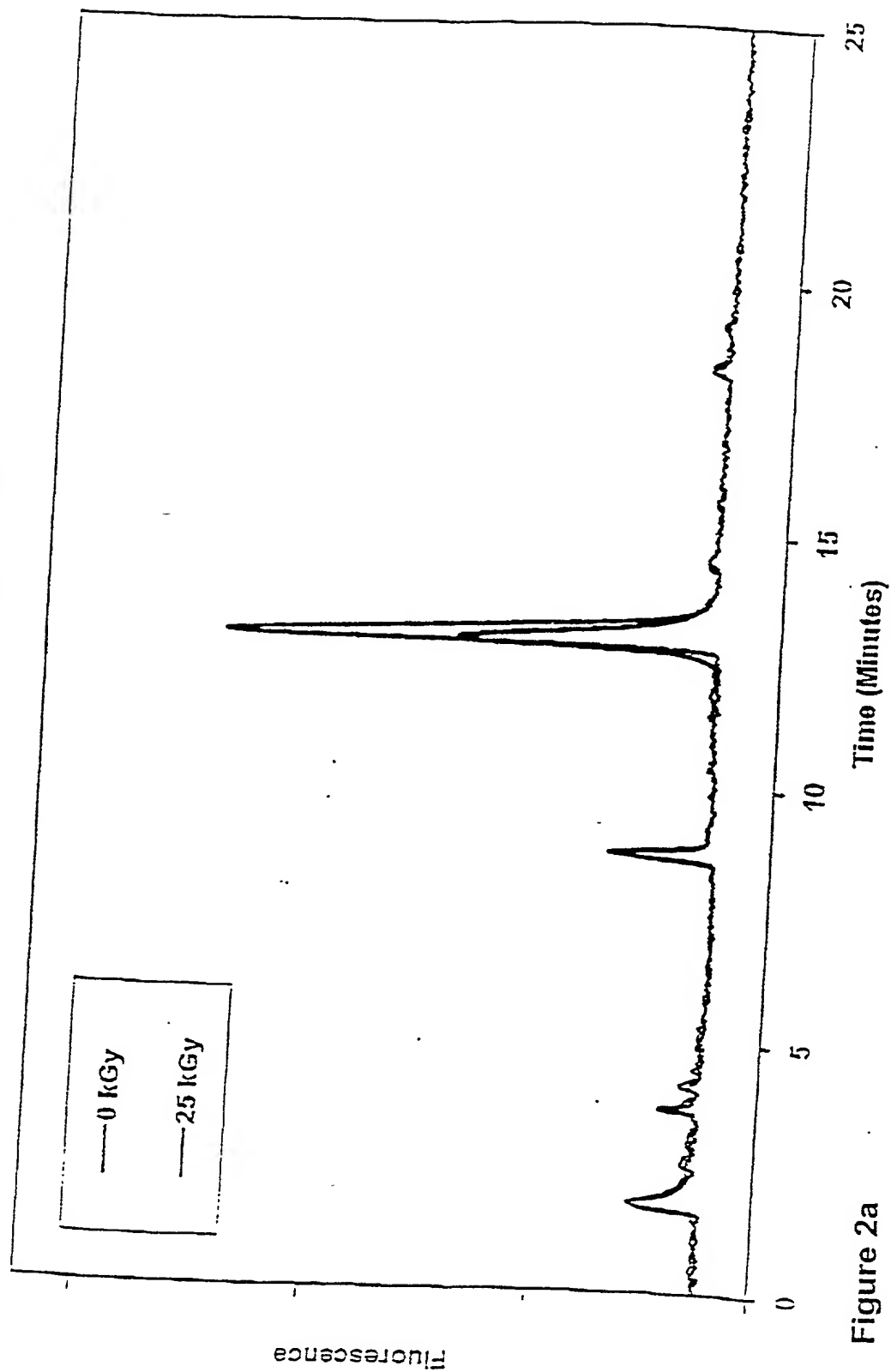


Figure 2a

**Gamma Irradiation of Hydrolyzed Heart Valve
Cusps in the Presence of PPG 400**

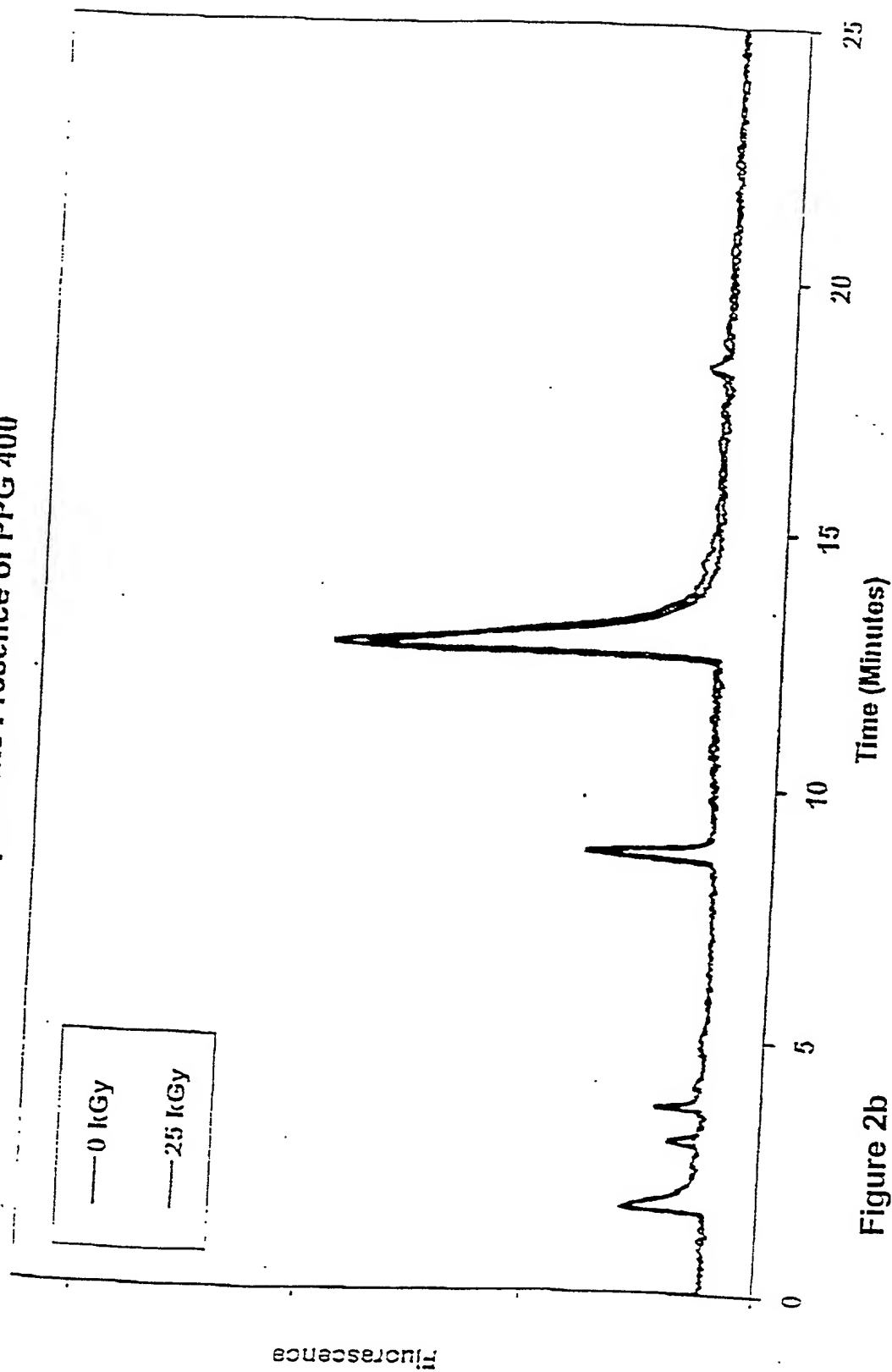


Figure 2b

Gamma Irradiation of Hydrolyzed Heart Valve Cusps
in the Presence of 50% DMSO

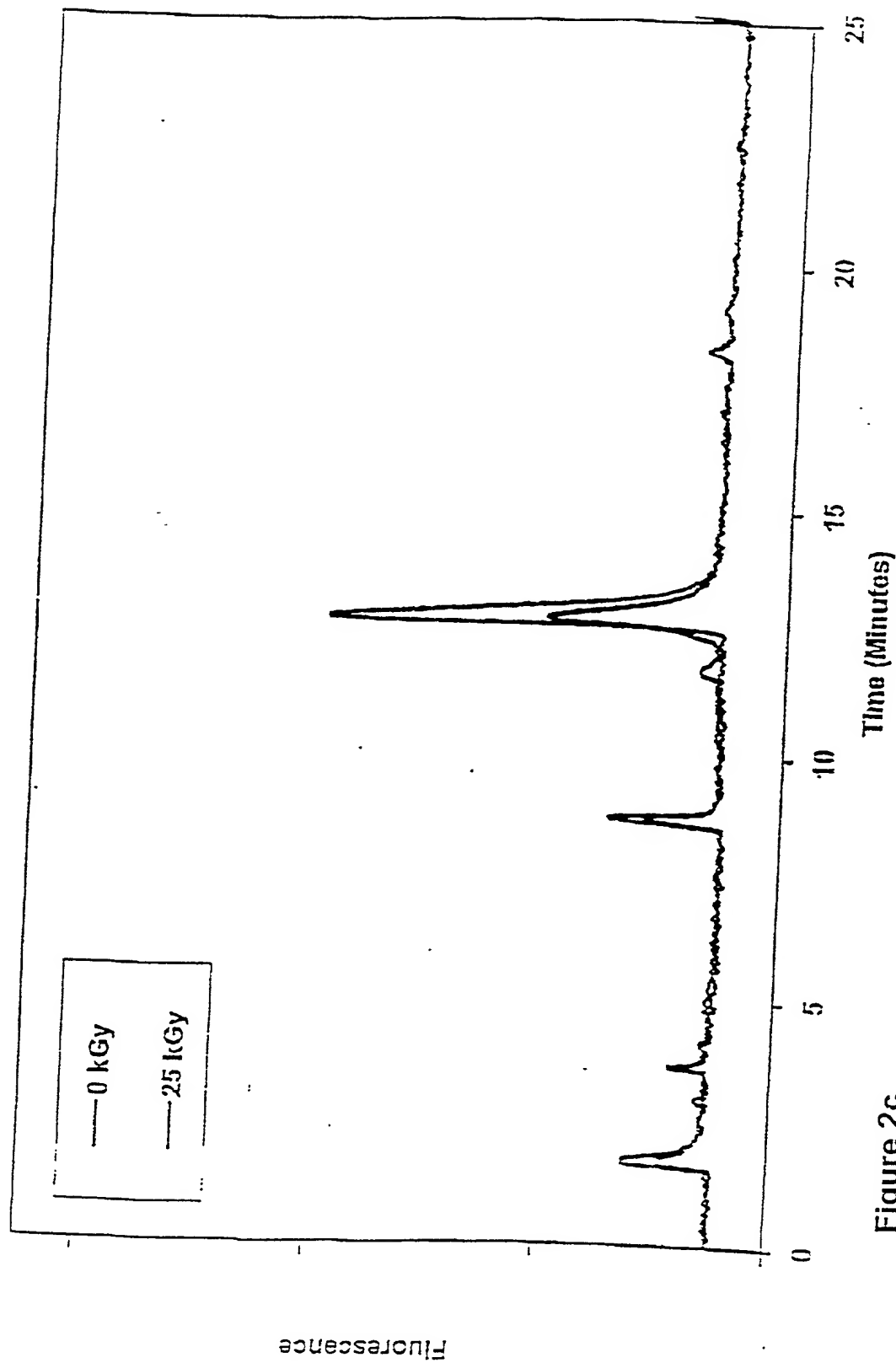


Figure 2c

Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the
Presence of 50% DMSO and a Stabilizer Mixture of 167 mM Ascorbate,
166 mM Coumaric Acid, and 100 mM n-Propyl Gallate

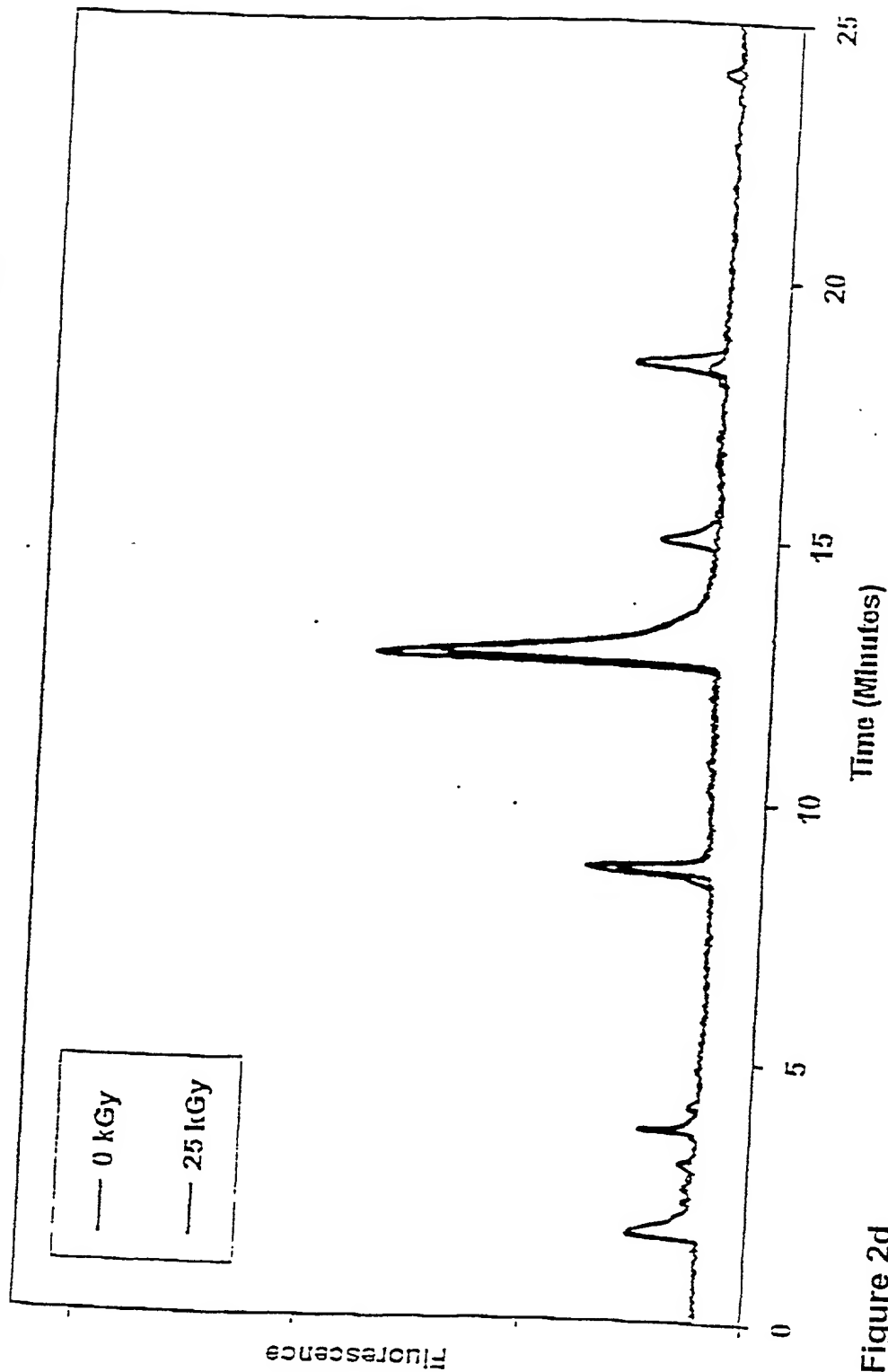
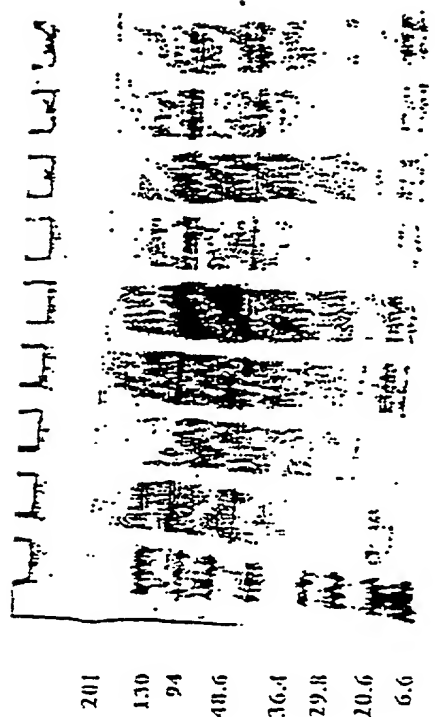


Figure 2d

Gamma Irradiation of Porcine Heart Valve Cusps in the Presence of Various Solvents

Reduced



1. Molecular Weight Markers

2. PBS, 0 kGy

3. PBS, 25 kGy

4. PPG400, 0 kGy

5. PPG400, 25 kGy

6. 50% DMSO, 0 kGy

7. 50% DMSO, 25 kGy

1 2 3 4 5 6 7 8 9

8. 50% DMSO and Cocktail of Ascorbate, Coumaric Acid, and n-Propyl Gallate, 0 kGy

9. 50% DMSO and Cocktail of Ascorbate, Coumaric Acid, and n-Propyl Gallate, 25 kGy

Figure 2e

Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the Presence of PBS

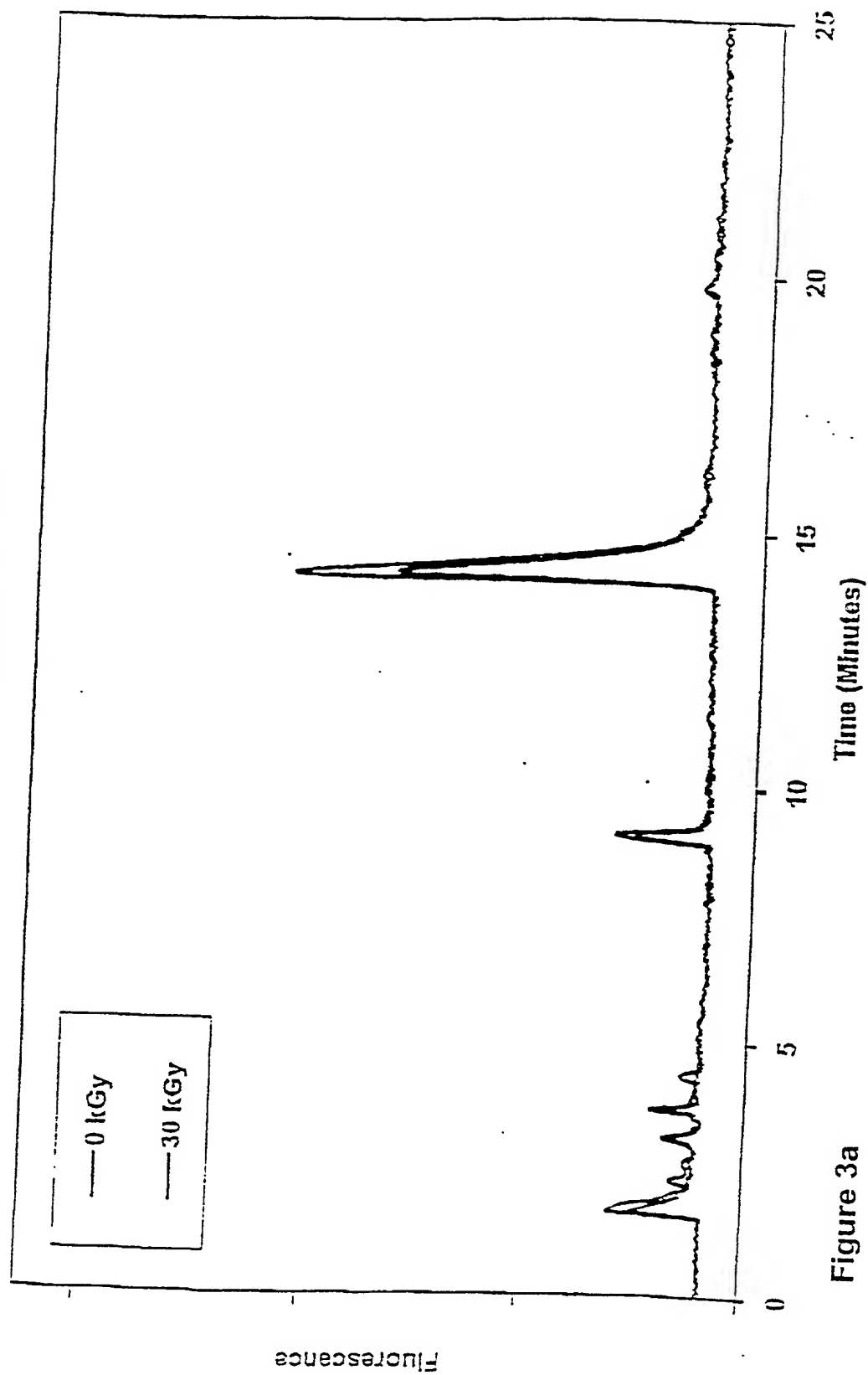


Figure 3a

Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the Presence of a Cryopreservative (Containing Approximately 20% DMSO)

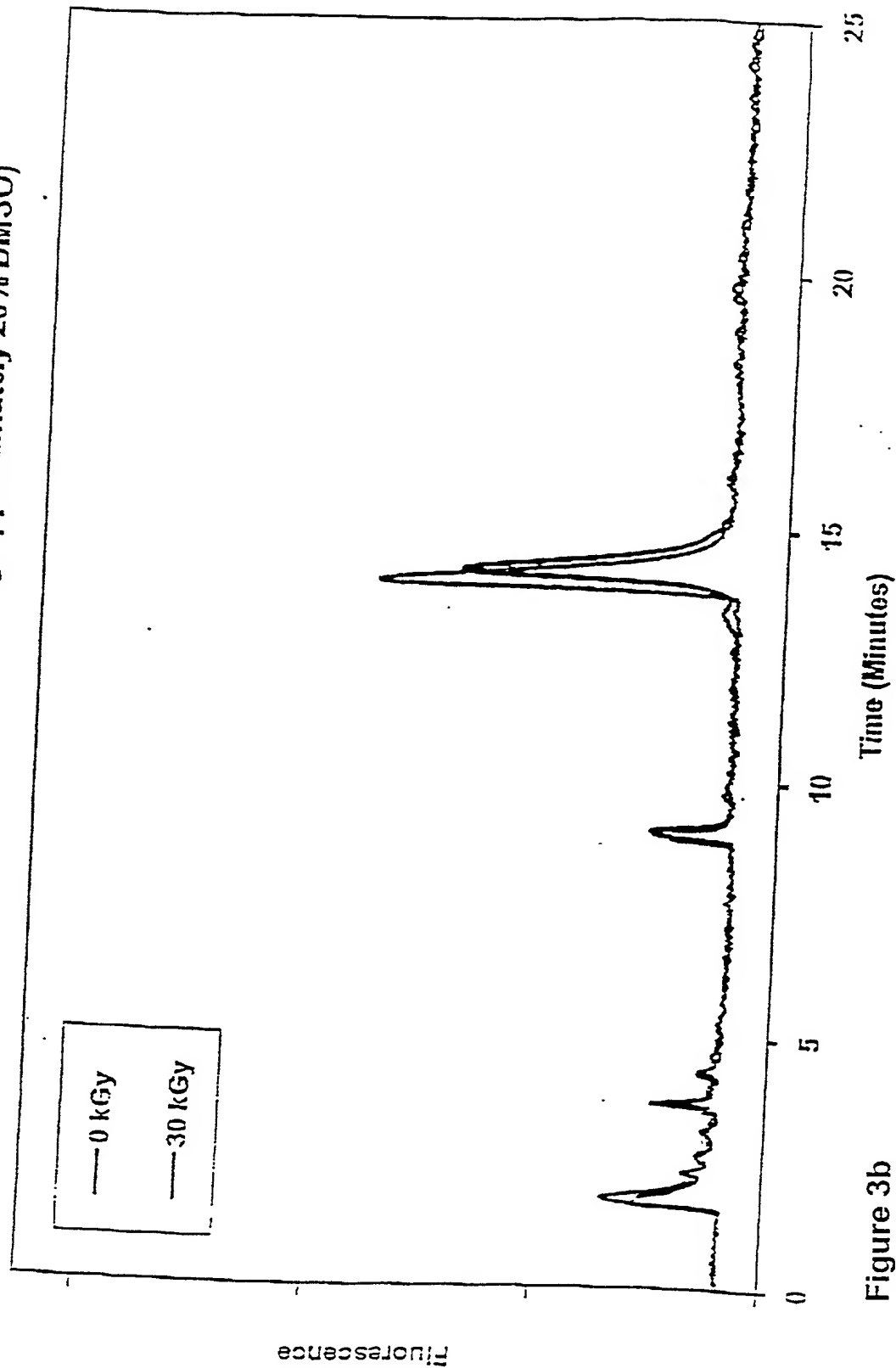


Figure 3b

Gamma Irradiation of Hydrolyzed Heart Valve
Cusps in the Presence of 50% DMSO

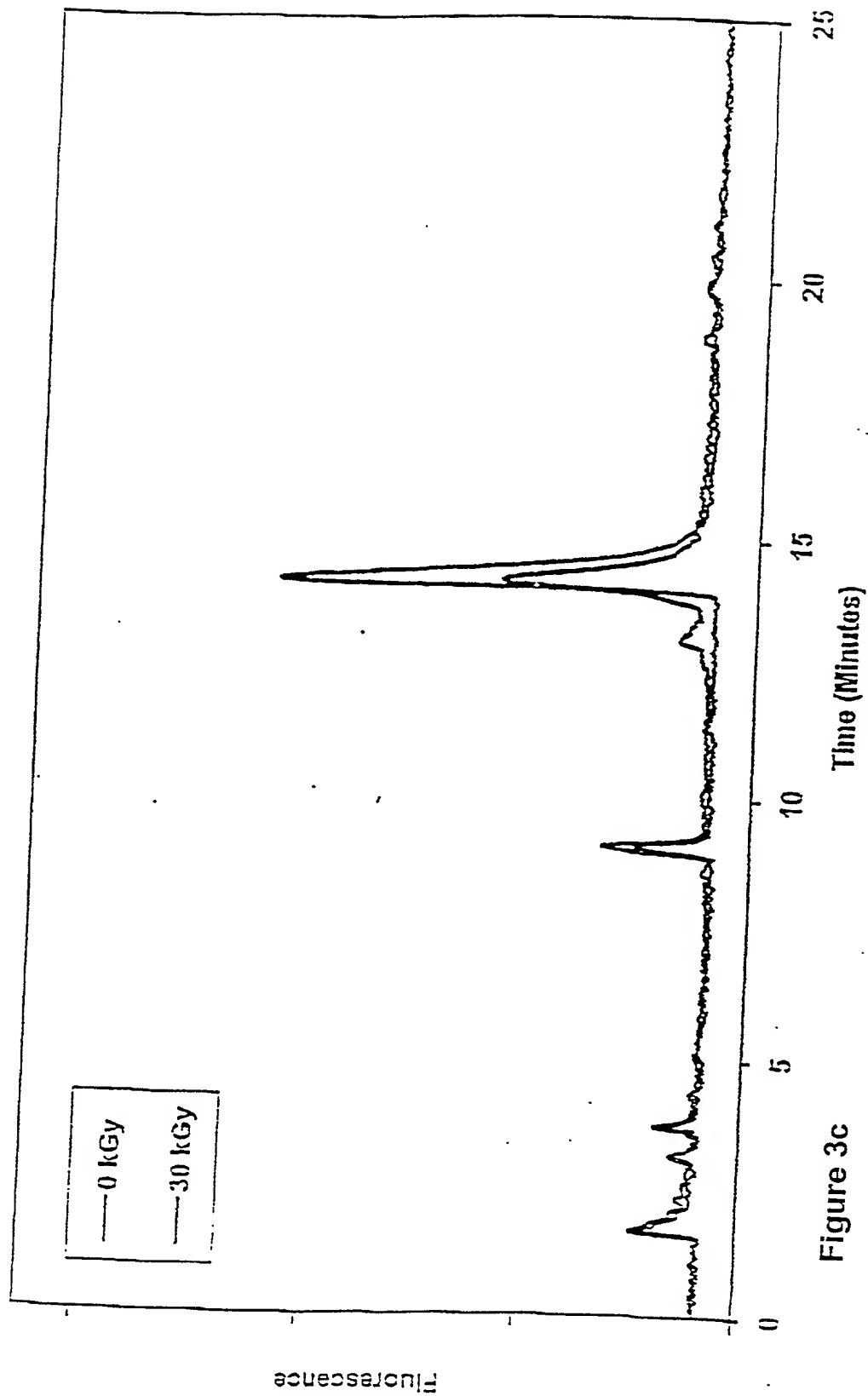


Figure 3c

Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the Presence of 50% DMSO and Ascorbate

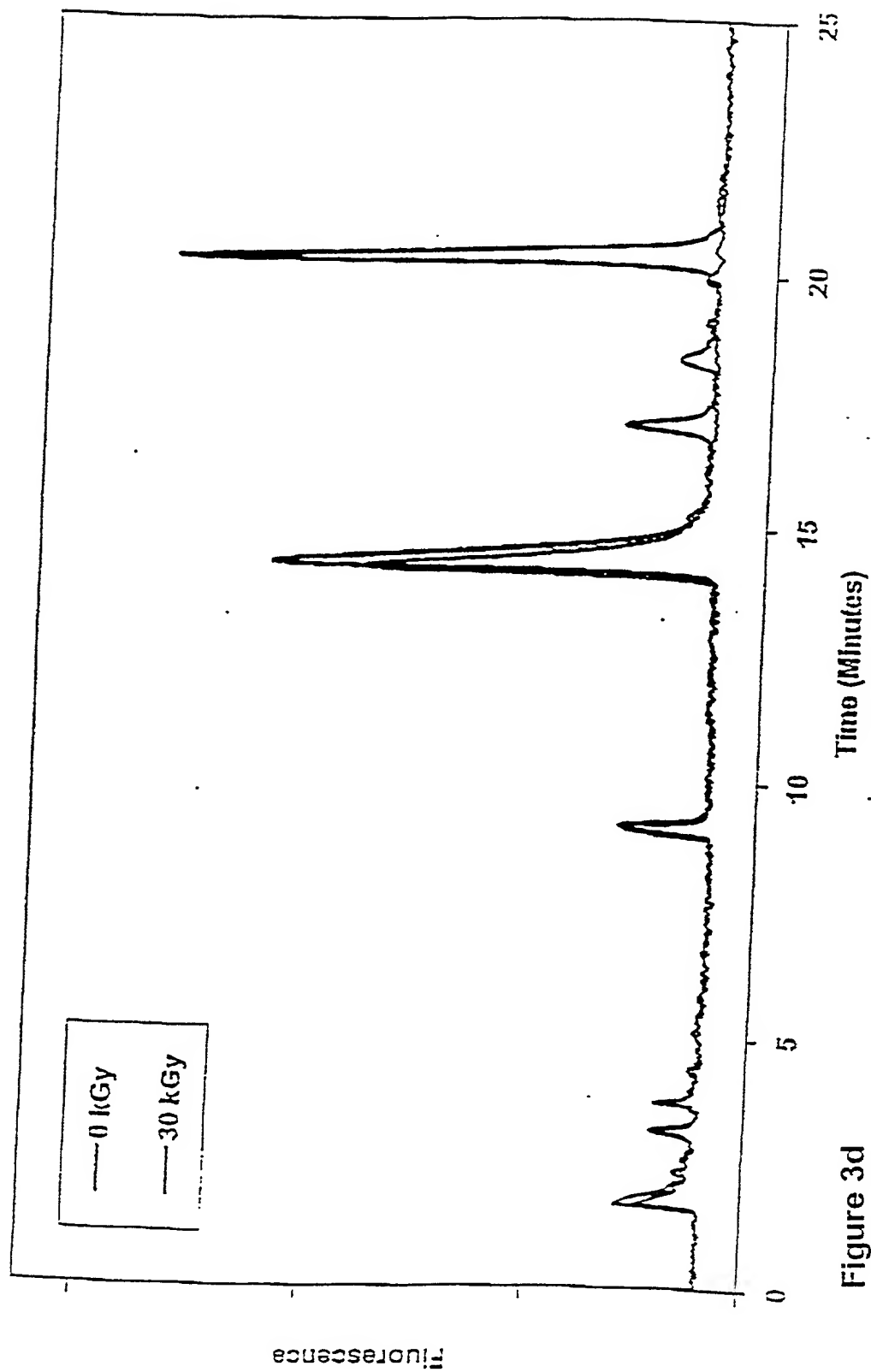


Figure 3d

Gamma Irradiation of Porcine Heart Valve Cusps in the Presence of Various Solvents

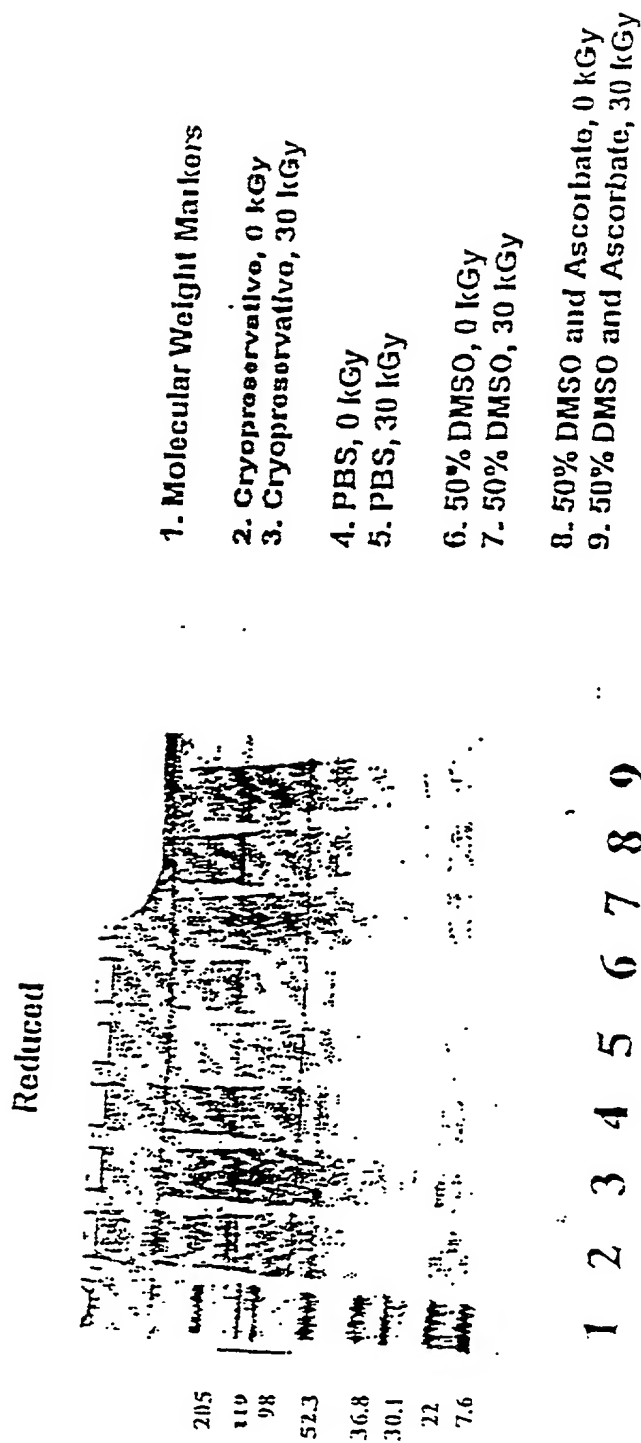


Figure 3e

Gamma Irradiation of Hydrolyzed Heart Valve Cusps in the Presence of PBS

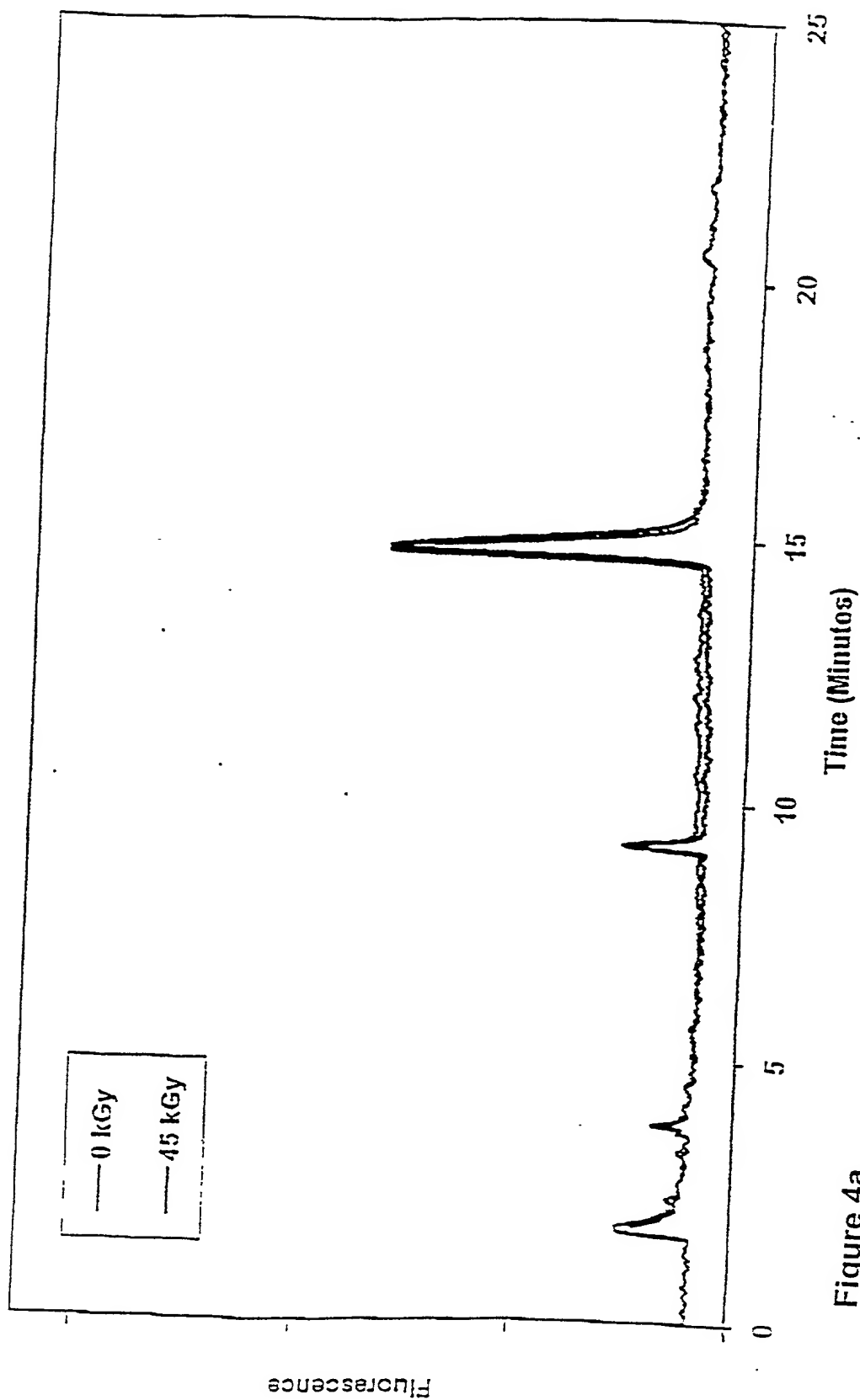


Figure 4a

**Gamma Irradiation of Hydrolyzed Heart Valve Cusps
in the Presence of PBS and Ascorbate**

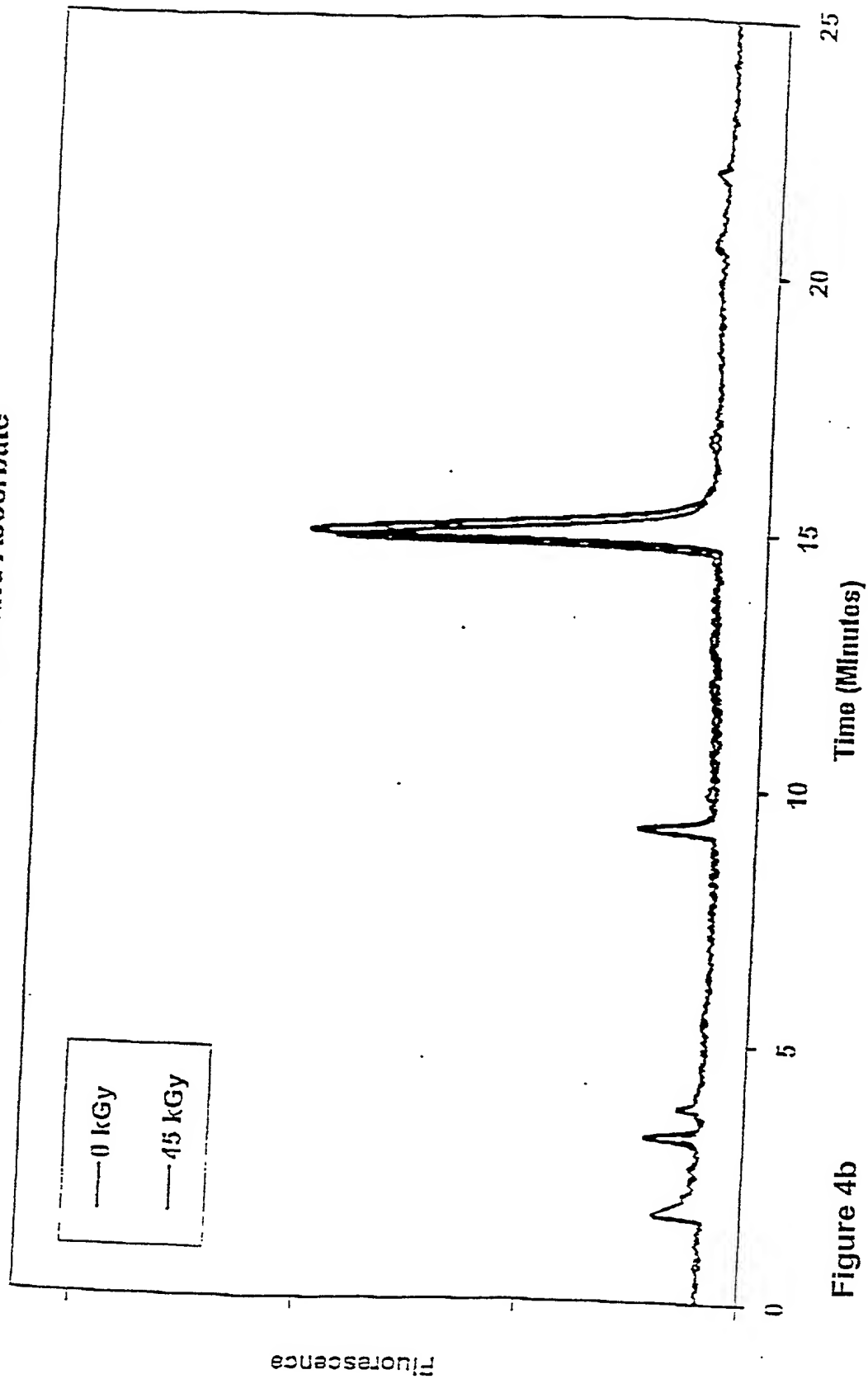


Figure 4b

Gamma Irradiation of Hydrolyzed Heart Valve Cusps
in the Presence of PPG 400

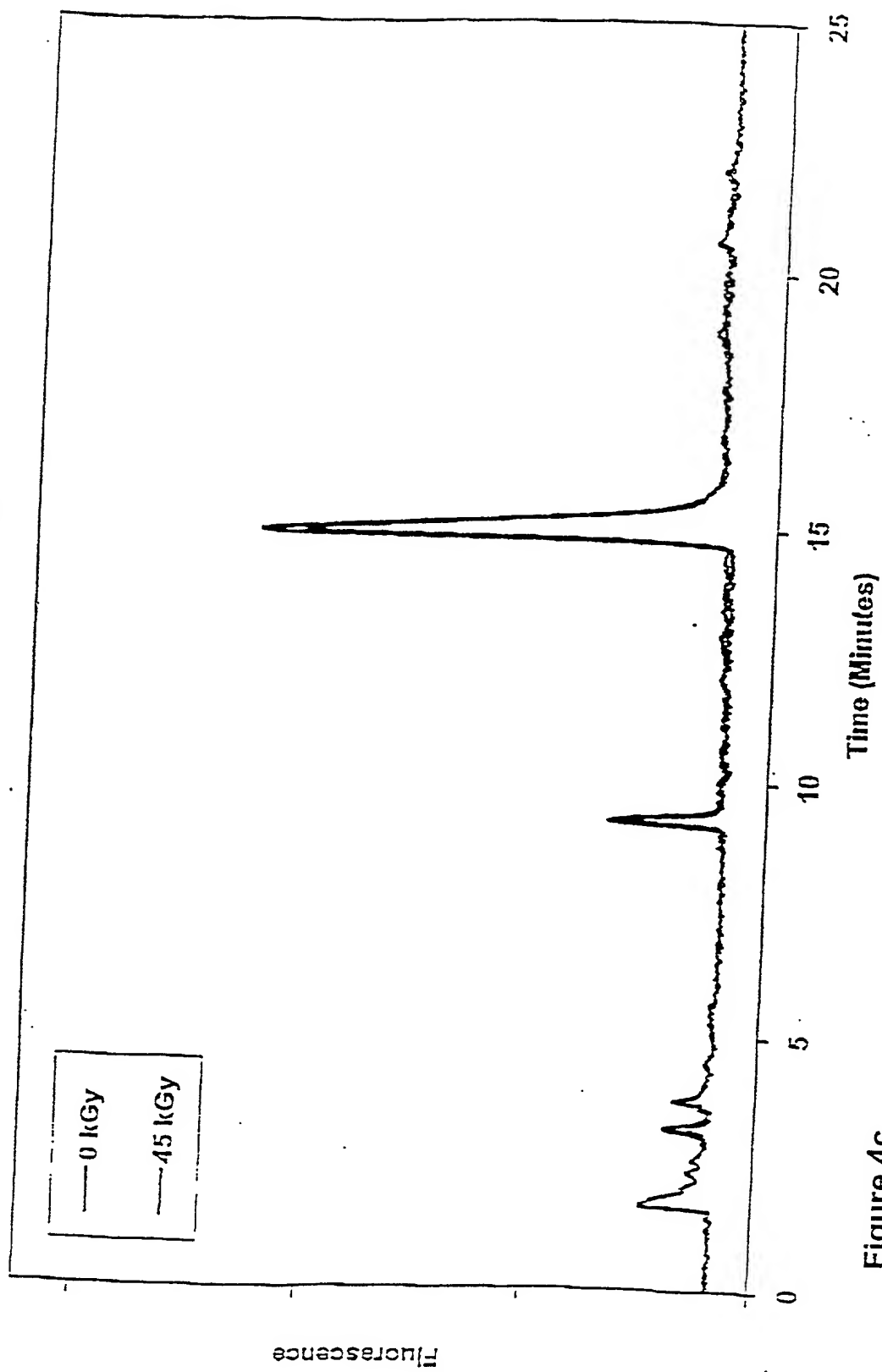


Figure 4c

Gamma Irradiation of Hydrolyzed Heart Valve Cusps
Dehydrated with PPG 400 and Rehydrated in the Presence of
PBS and Ascorbate

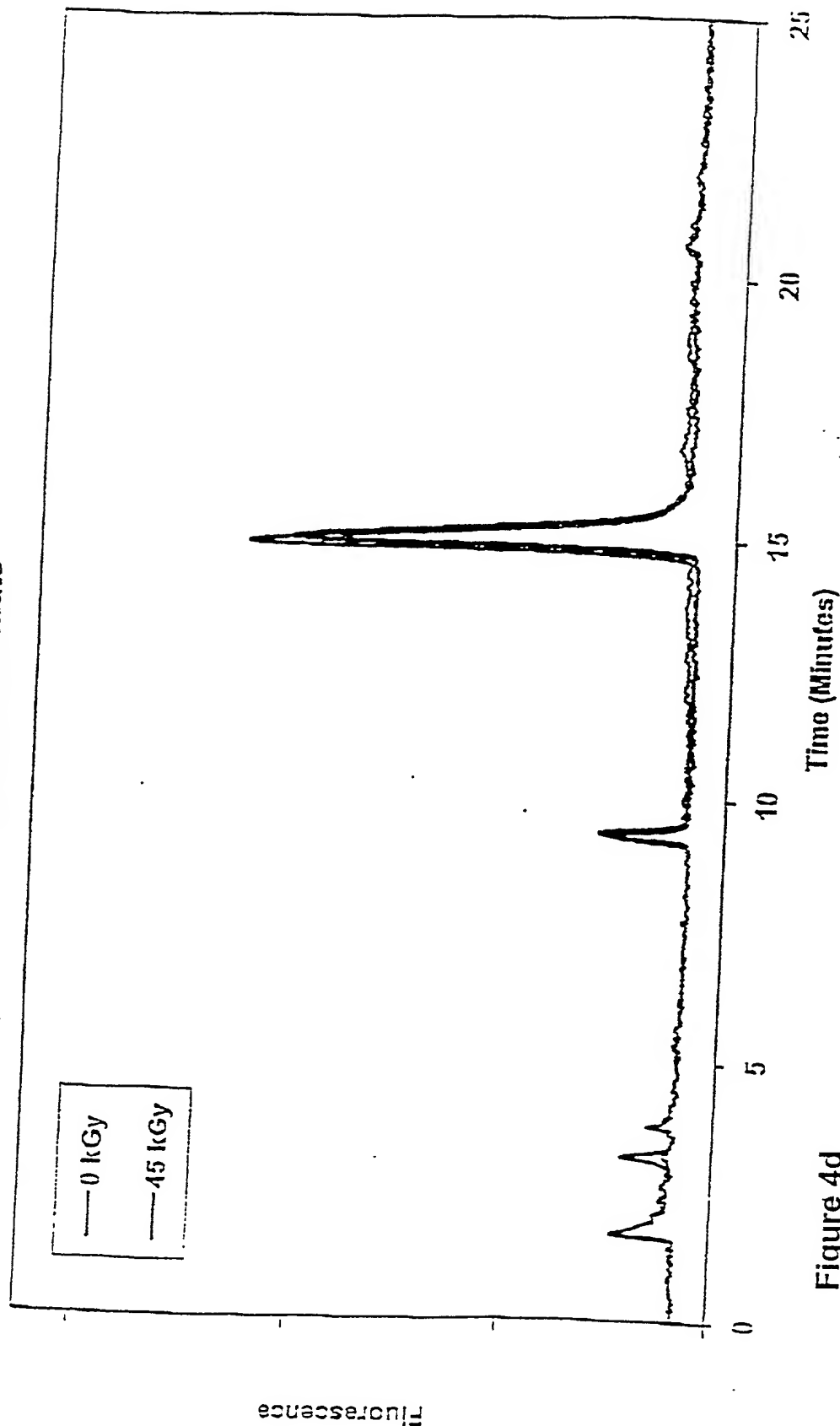


Figure 4d

Gamma Irradiation of Hydrolyzed Heart Valve Cusps
in the Presence of 50% DMSO

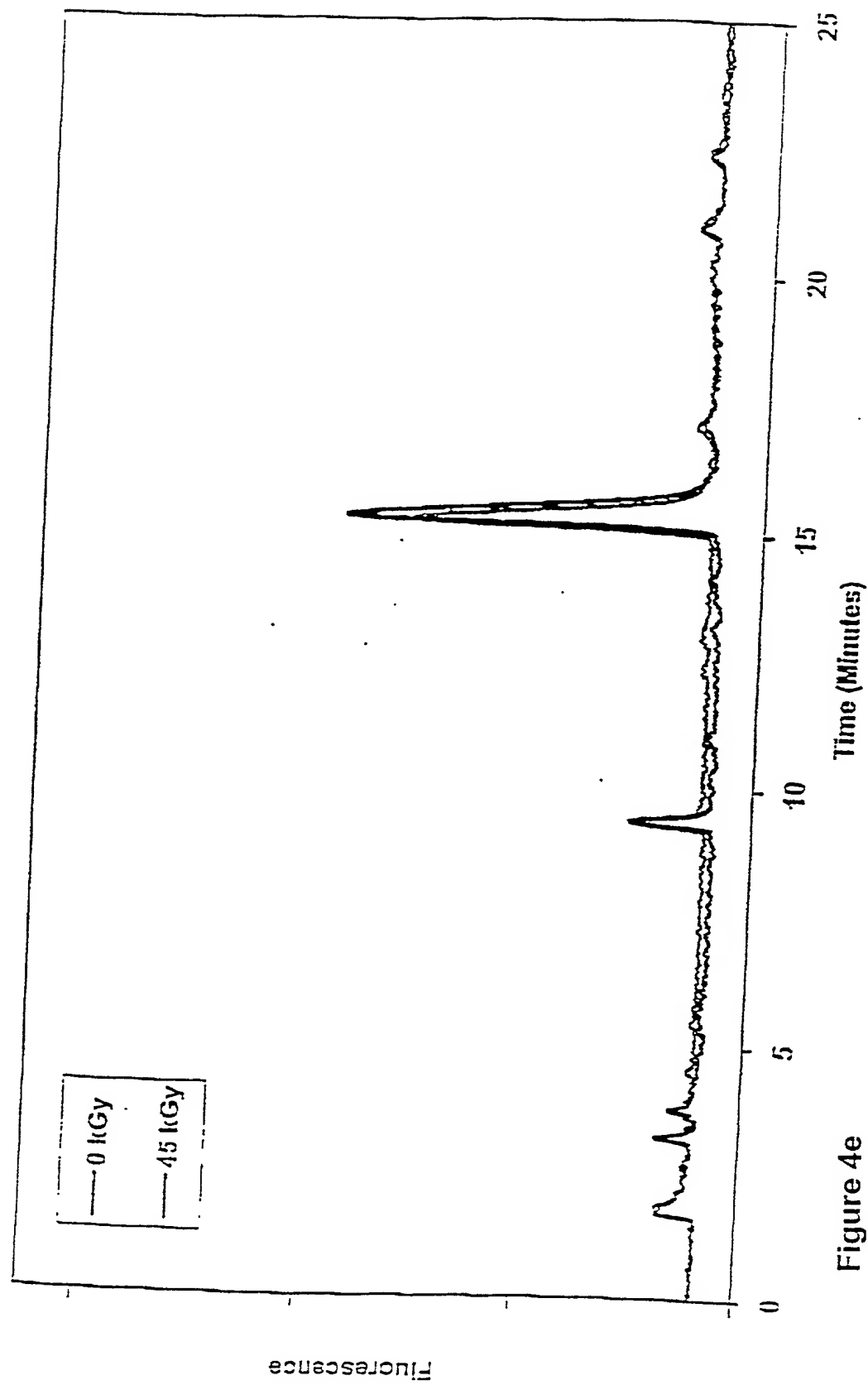


Figure 4e

Gamma Irradiation of Hydrolyzed Heart Valve Cusps
in the Presence of 50% DMSO and Ascorbate

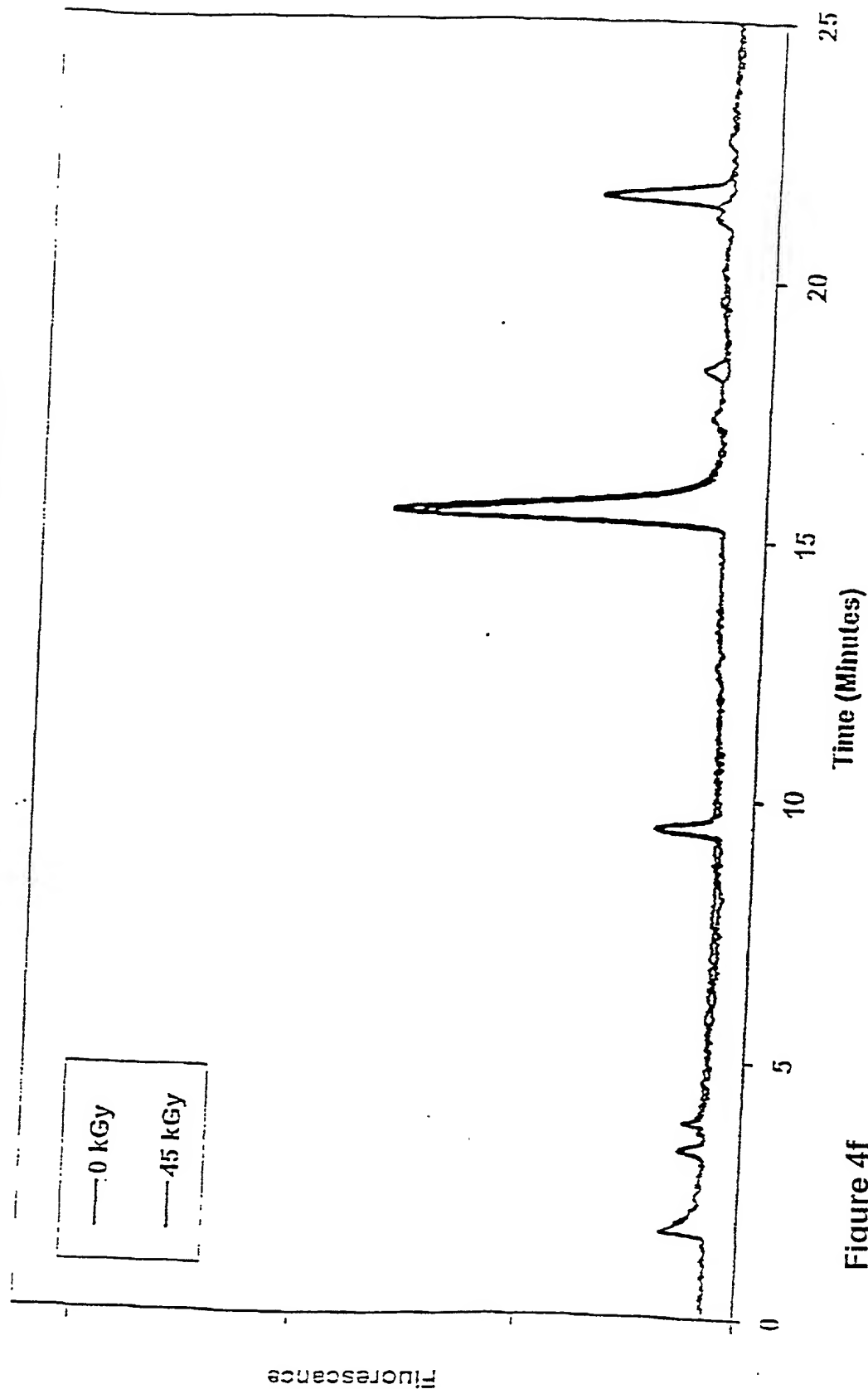


Figure 4f

Valve Cusps in the Presence of Various Solvents

Gamma Irradiation of Porcine Heart

1. Molecular Weight Markers

2. PBS, 0 kGy

3. PBS, 45 kGy

4. PBS and Ascorbate, 0 kGy

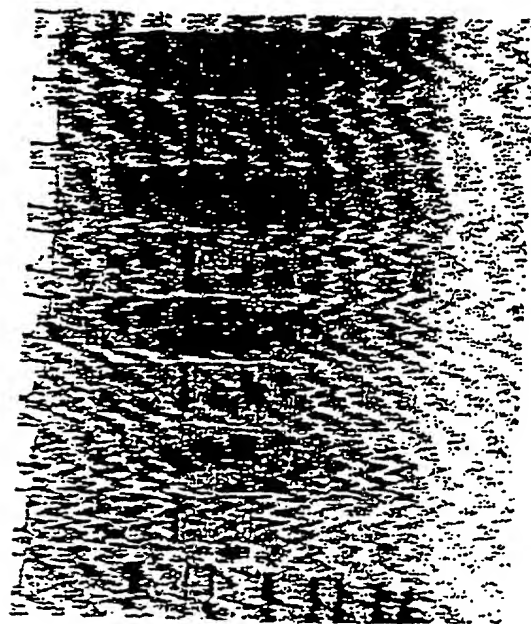
5. PBS and Ascorbate, 45 kGy

6. PPG400, 0 kGy

7. PPG400, 45 kGy

8. Dehydrated in PPG400 and Rehydrated
with PBS and Ascorbate, 0 kGy

9. Dehydrated in PPG400 and Rehydrated
with PBS and Ascorbate, 45 kGy



205
119
98
52.3
36.8
30.1
22
7.6

1 2 3 4 5 6 7 8 9

Figure 4g

Valve Cusps in the Presence of Various Solvents

Gamma Irradiation of Porcine Heart

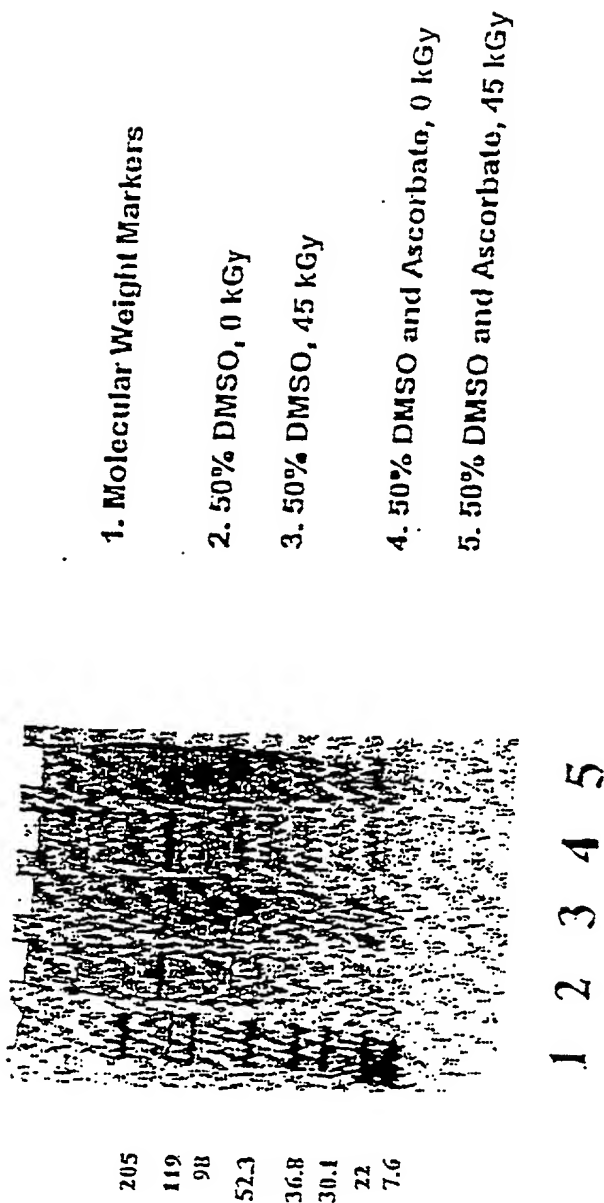
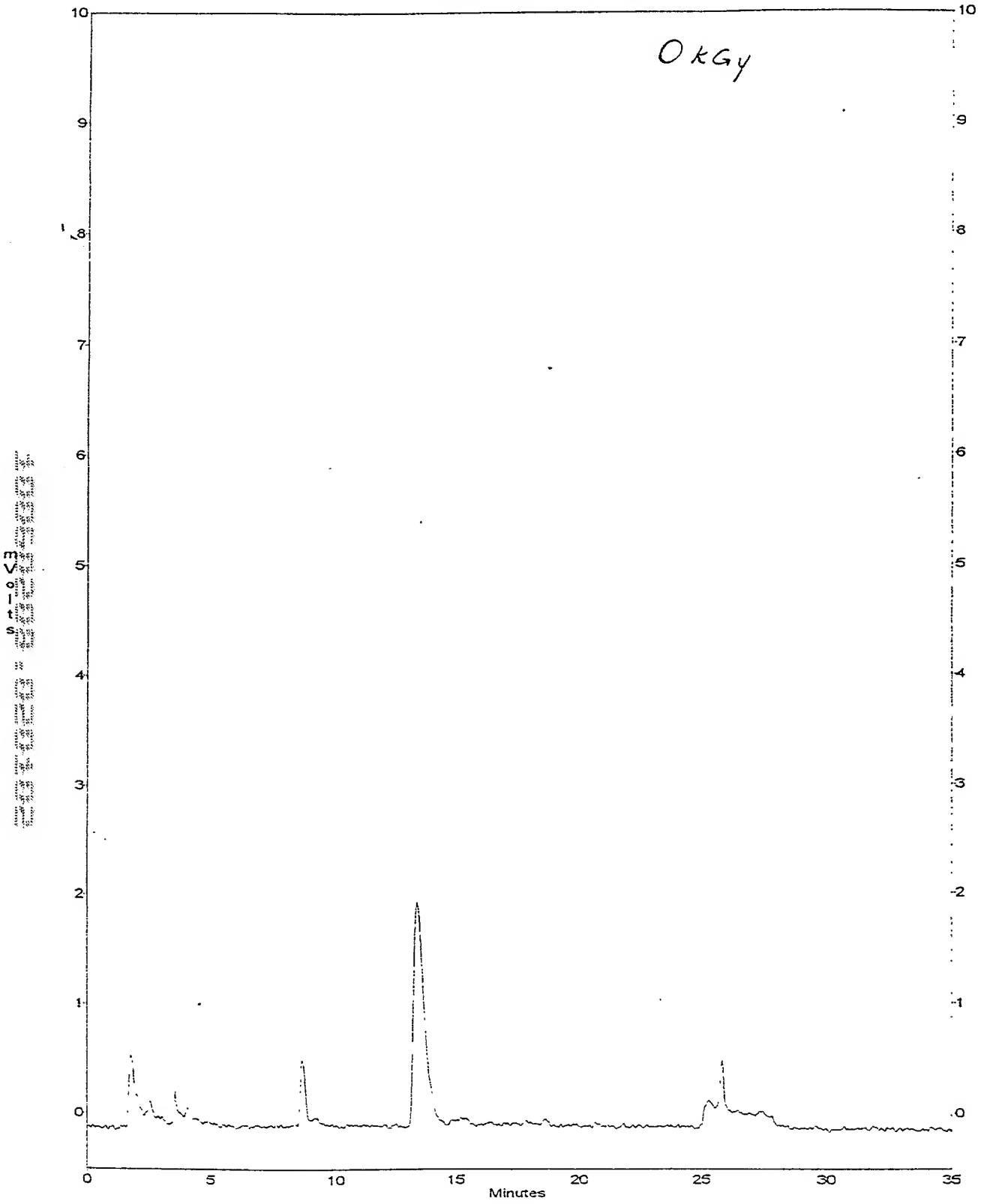


Figure 4h

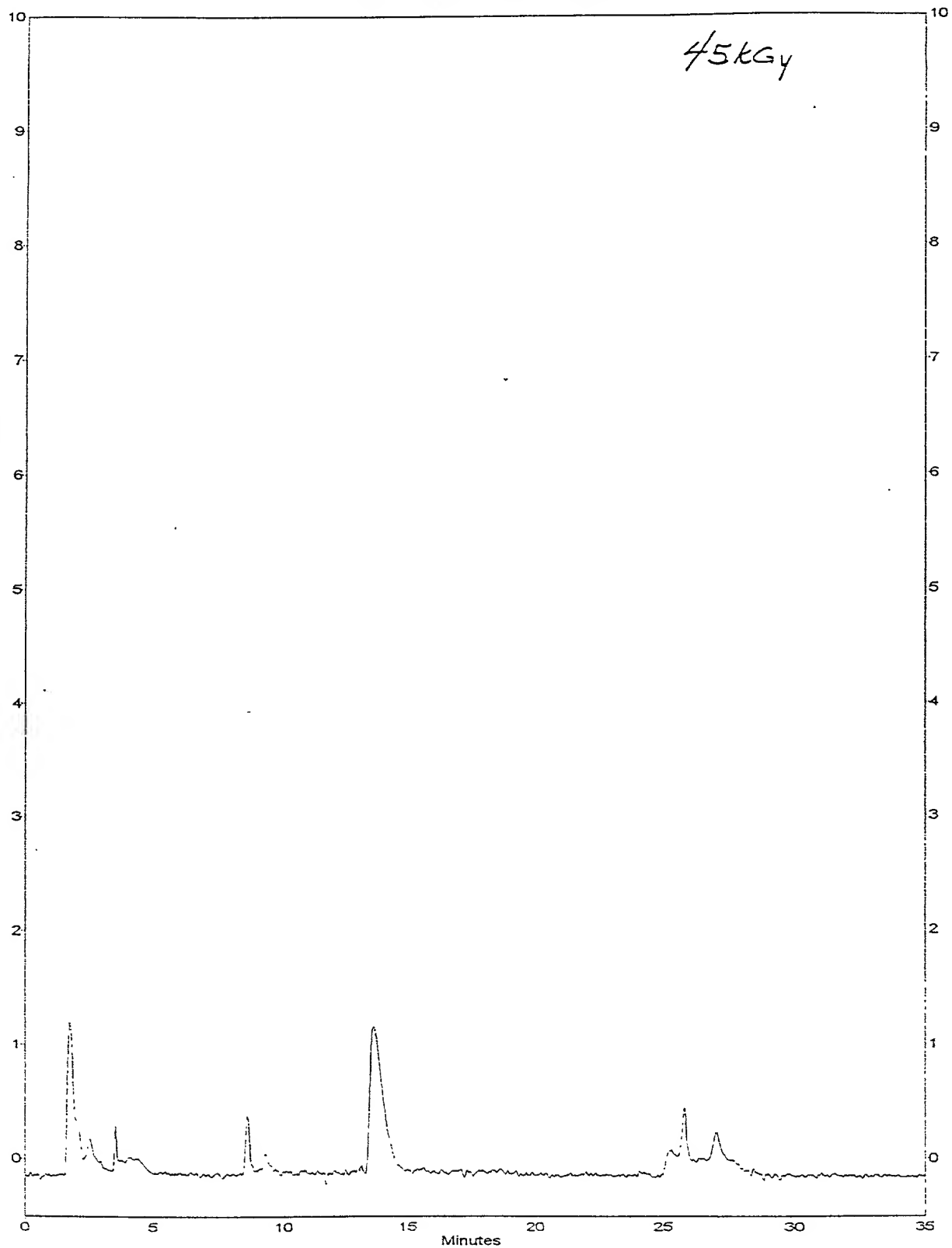
[illegible]

AED = 100 mM Ascorbate, 22 mM Ergothionine, 100 mM Deferoxamine

c:\class-vp\methods\1028, Channel B



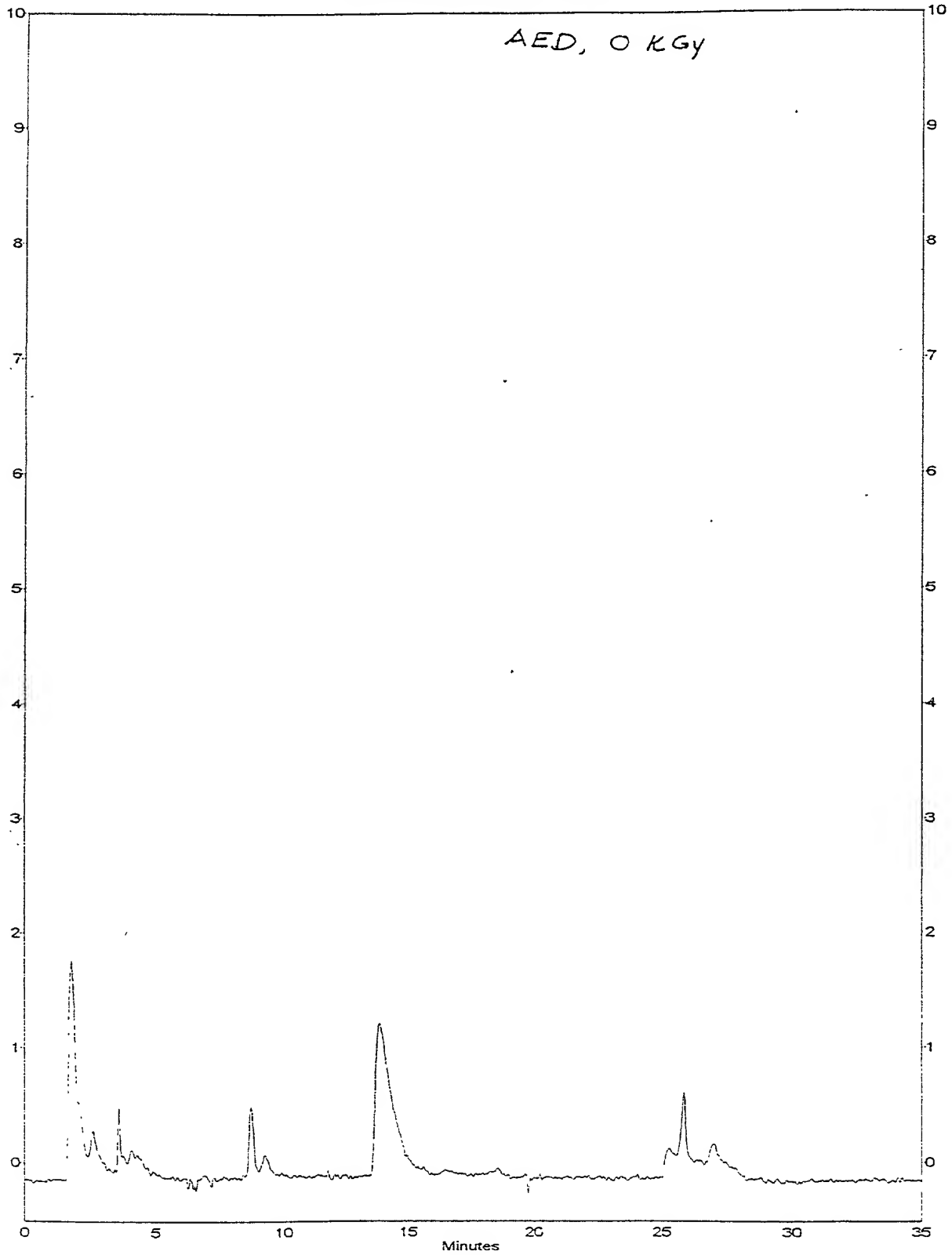
c:\class-vp\methods\1029, Channel B



mV

25

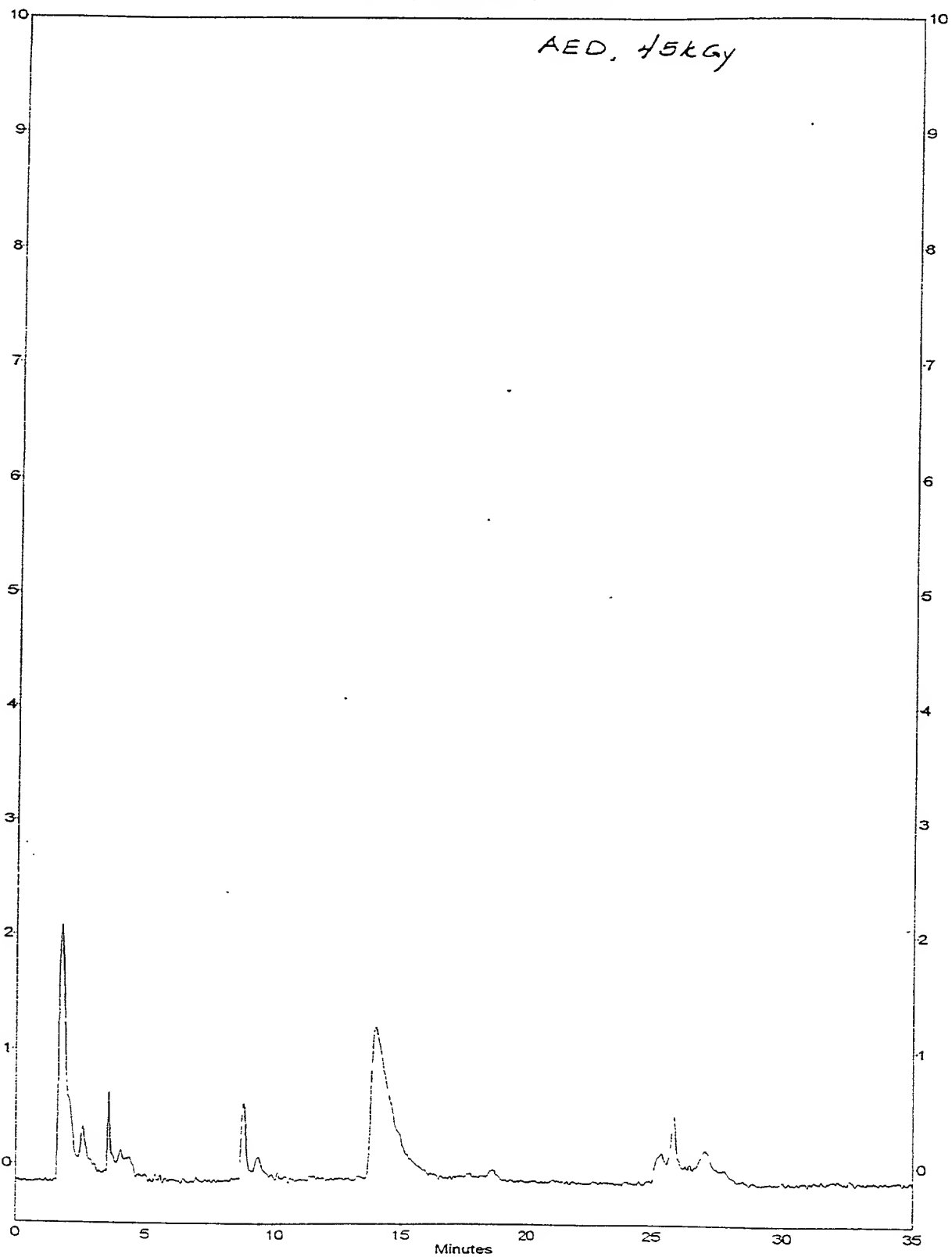
c:\class-vpl\methods\1030, Channel B



Counts

50

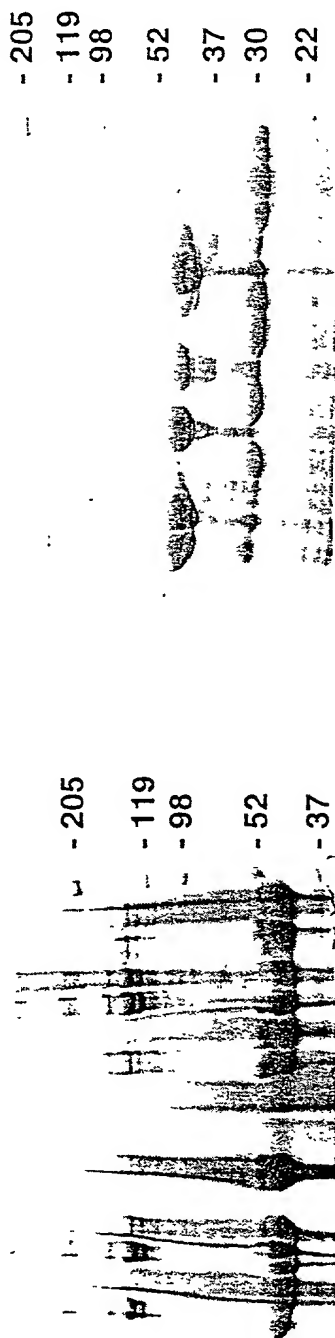
c:\class-vp\methods\1031, Channel B



Volts

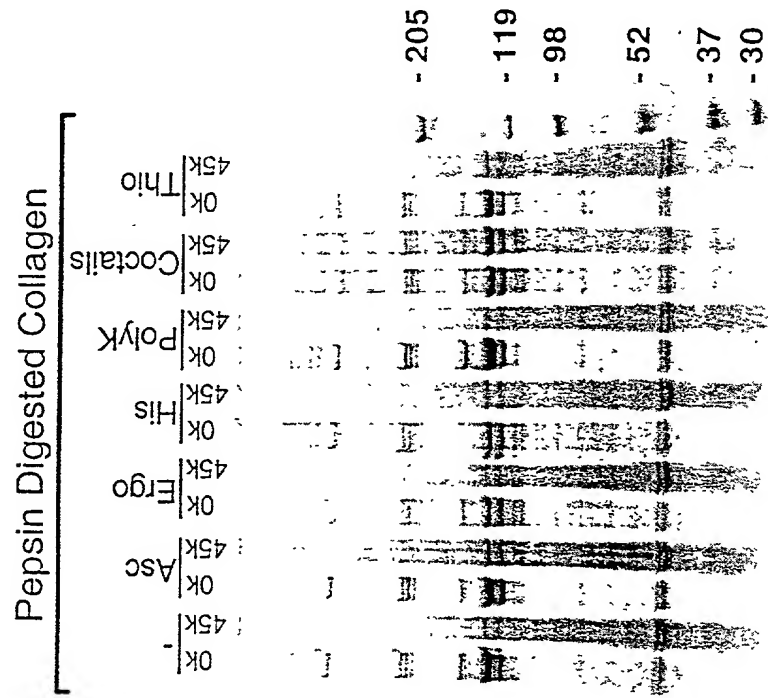
SE

γ -irradiation of Freeze-Dried Porcine ACL in the Presence of Antioxidants at 4°C



67

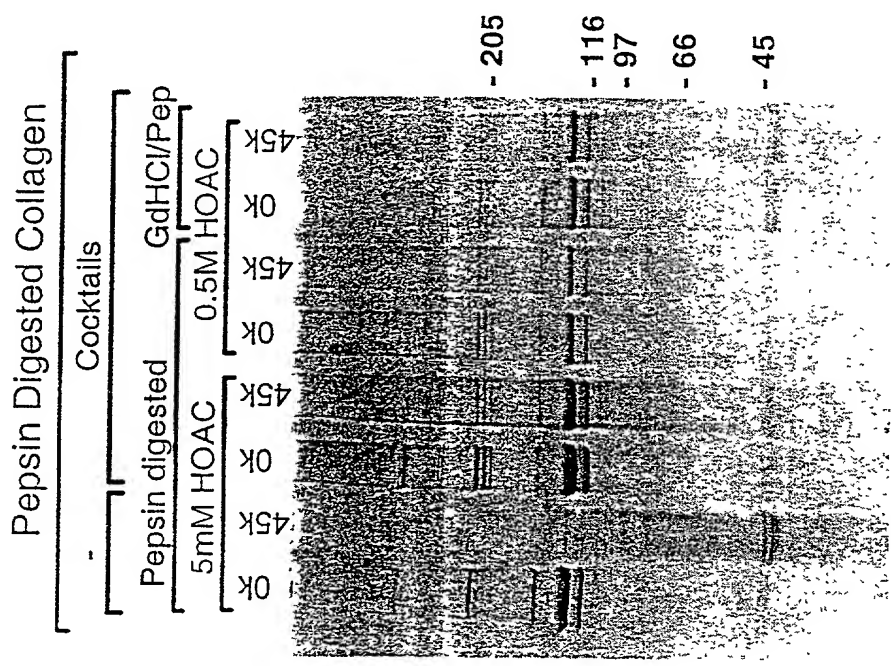
Pepsin-Digested Collagen Isolated from Irradiated Freeze-Dried Porcine ACL in the Presence of Antioxidants at 4°C 1.667kGy/hr



Cocktails: PPG/presoak; 100µM trolox, 100mM ascorbic acid, 100mM lipoic acid, 100mM n-propyl gallate

CS

Effect of Antioxidants on the Stability of Radiolabelled Porcine ACL in the Presence of Antioxidants at 4°C 1.667kGy/hr

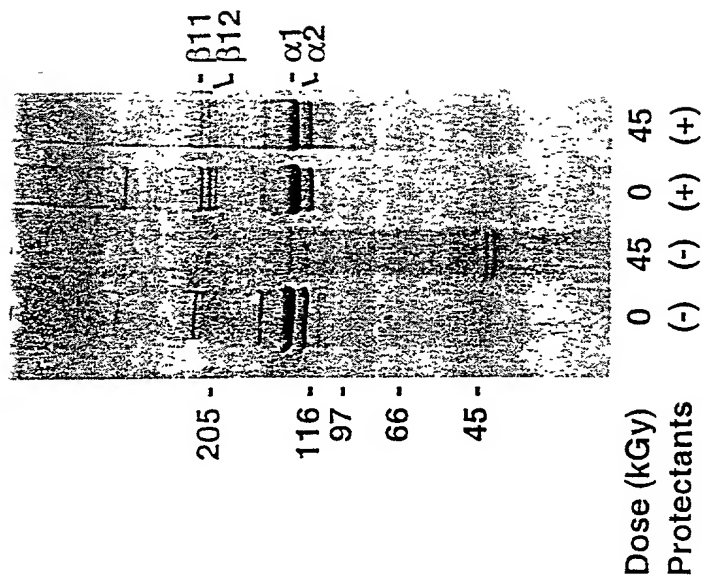


Cocktails: PPG/presoak; 100µM trolox, 100mM ascorbic acid, 100mM lipoic acid, 100mM n-propyl gallate

6C

Porcine Brainstem Cellulose Isolated from Irradiated Freeze-Dried Porcine ACL in the

presence of various antioxidants at 4°C 1.00 / kGy/hr



Protectants: PPG/presoak;
100μM trolox,
100mM ascorbic acid,
100mM lipoic acid, and
100mM n-propyl gallate

6D

Guanidine Extraction of Freeze-dried ACL's

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

205—
119—
98—
52—
37—
30—
22—
8—

1 = 0 kGy
2 = 45 kGy
3 = 200 mM Ascorbate, 0 kGy
4 = 200 mM Ascorbate, 45 kGy
5 = 100 mM Thiourea, 0 kGy
6 = 100 mM Thiourea, 45 kGy
7 = 200 mM Histidine in PBS, 0 kGy
8 = 200 mM Histidine in PBS, 45 kGy

9 = 500 mM Trehalose, 0 kGy
10 = broad range markers
11 = 500 mM Trehalose, 45 kGy
12 = 5 mg/ml Ergothionine, 0 kGy
13 = 5 mg/ml Ergothionine, 45 kGy
14 = 10 mM Poly-lysine, 0 kGy
15 = 10 mM Poly-lysine, 45 kGy
16 = PPG, then cocktail (100µM trolox, 100mM
courmeric acid, 100mM lipoic acid, 100mM
propyl gallate), 0 kGy
17 = PPG, then cocktail, 45 kGy

66

Purified GAG/Proteoglycans from Irradiated ACL



205 —
119 —
98 —
52 —
37 —
30 —
22 —

Lane:

- 1 = Recombinant Human Decorin
- 2 = No Scavengers, 0 kGy
- 3 = No Scavengers, 45 kGy
- 4 = 200 mM Ascorbate, 0 kGy
- 5 = 200 mM Ascorbate, 45 kGy
- 6 = 100 mM Thiourea, 0 kGy
- 7 = 100 mM Thiourea, 45 kGy
- 8 = 200 mM Histidine in PBS, 0 kGy
- 9 = 200 mM Histidine in PBS, 45 kGy

Lane:

- 1 = 500 mM Trehalose, 0 kGy
- 2 = 500 mM Trehalose, 45 kGy
- 3 = 5 mg/ml Ergothionine, 0 kGy
- 4 = 5 mg/ml Ergothionine, 45 kGy
- 5 = 10 mM Poly-lysine, 0 kGy
- 6 = 10 mM Poly-lysine, 45 kGy
- 7 = PPG Pretreatment, then cocktail, 0 kGy
- 8 = PPG Pretreatment, then cocktail, 45 kGy
- 9 = Recombinant Human Decorin

6F

DEAE Chromatography of Porcine ACL Irradiated in Cryopreservative ± Ascorbate at -80°C at 5.1 kGy/h

Edmonton Cryopreservative

FC Regulated Quick

Modified VS55

Regulated Quick

205 —
119 —
98 —
52 —
37 —
30 —
22 —
8 —

0 0 50 0 50 0 50 0 50
- - - + + - - + +

0 50 0 50 0 50 0 50
- - + + - - + +

Edmonton CP: M199, 10% FCS, 2 M DMSO, Pen-Strep
Modified VS55: 100 mM trehalose, 15 mM KH_2PO_4 , 42 mM K_2HPO_4 , 15 mM KCl, 10 mM NaHCO_3 , 150 mM mannitol, 24.2% DMSO,
16.8% 1,2-propanediol, 14% formamide

FC: fresh ACL control

Quick Freeze: dry-ice ethanol bath

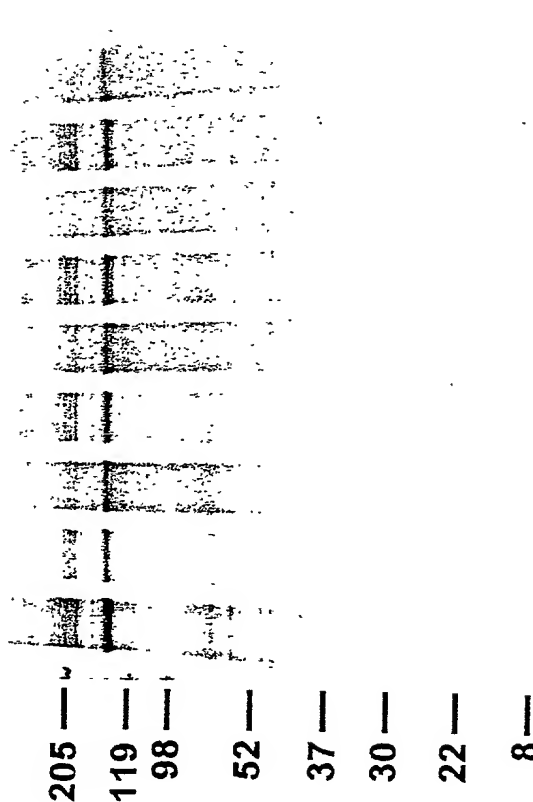
Regulated Freeze: decrease in temp. of 1°C/min to -56-59°C, then placed in -80°C

EA

Guanidine Extract of Porcine ACL Irradiated in Cryopreservative ± Ascorbate at -80°C at 5.1 kGy/h

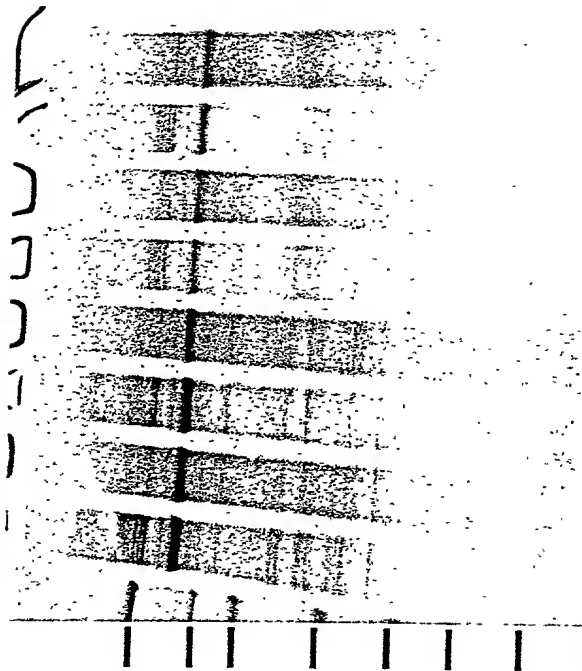
Edmonton Cryopreservative

FC Quick Regulated



Modified VS55

Quick Regulated



0 0 50 0 50 0 50 0 50
- - - + + - - + +

Dose (kGy)
Ascorbate

Edmonton CP: M199, 10% FCS, 2 M DMSO, Pen-Strep
Modified VS55: 100 mM trehalose, 15 mM KH_2PO_4 , 42 mM K_2HPO_4 , 150 mM mannitol, 24.2% DMSO,
16.8% 1,2-propanediol, 14% formamide

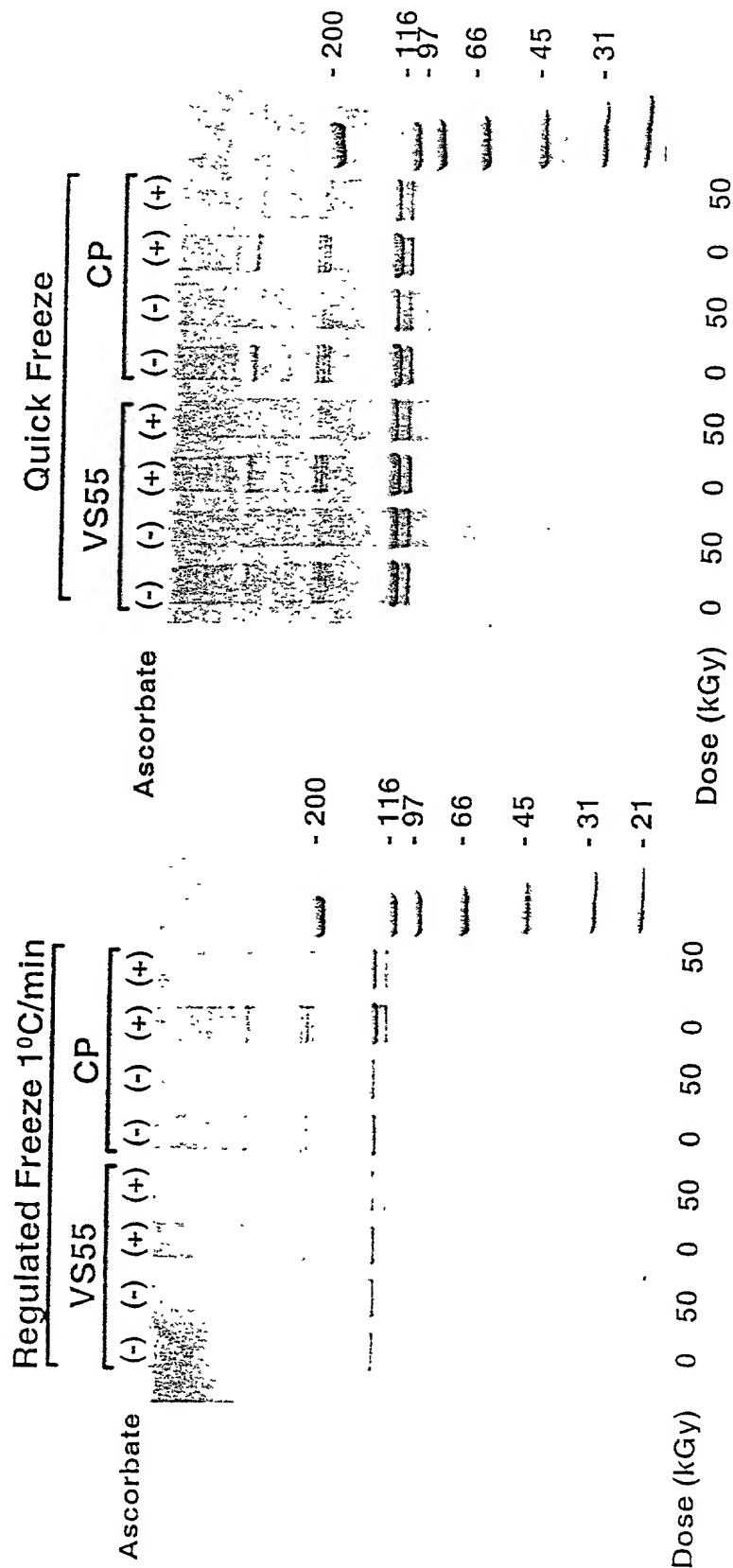
FC: fresh ACL control

Quick Freeze: dry-ice ethanol bath

Regulated Freeze: decrease in temp. of 1°C/min to -56-59°C, then placed in -80°C

78

Pepsin-Digested Collagen Isolated from Irradiated Porcine ACL in the Presence of Ascorbate at -80°C 5.1kGy/hr

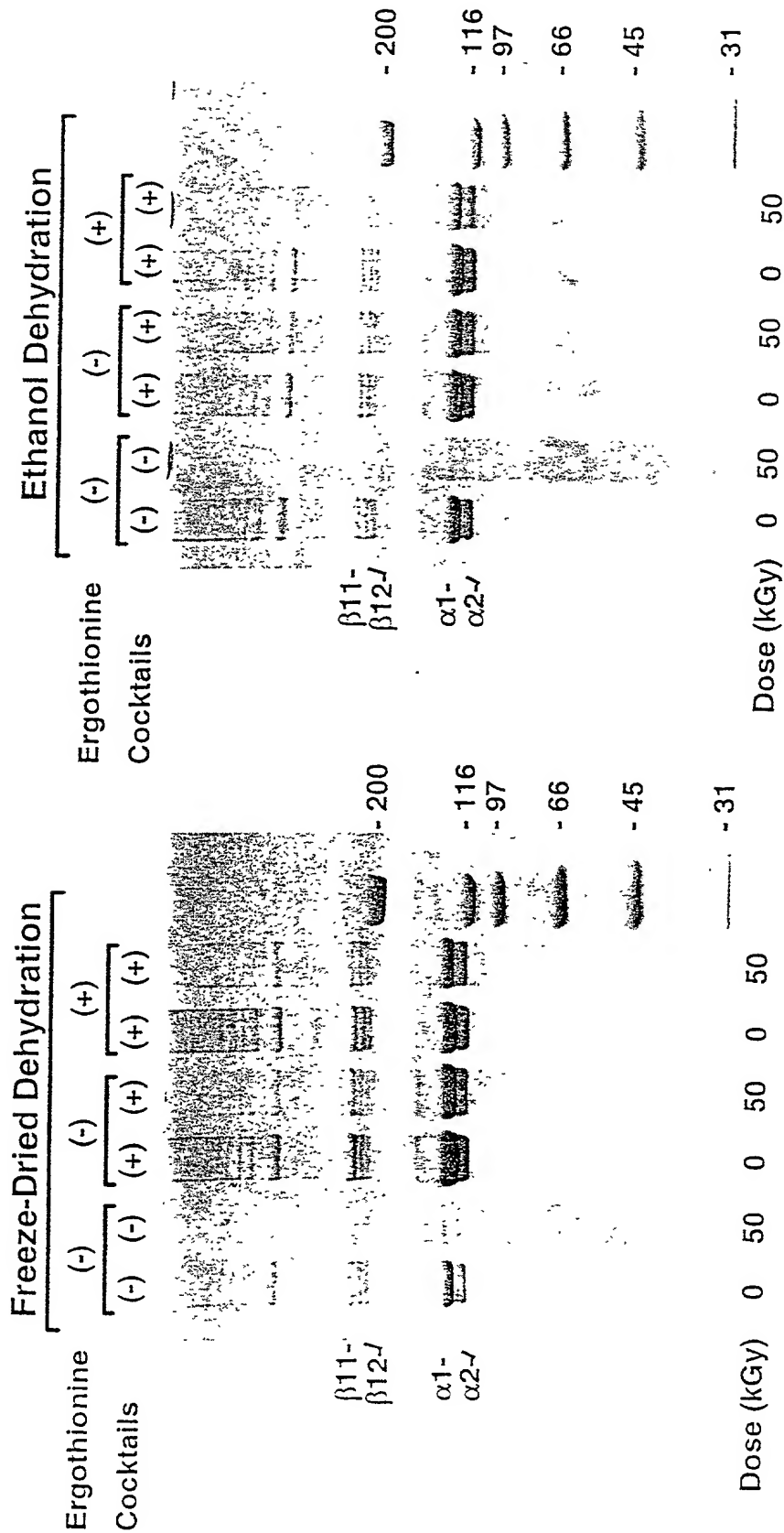


VS55: 100mM trehalose, 15mM KH_2PO_4 , 42mM K_2HPO_4 , 15mM KCl, 10mM NaHCO_3 , 150mM mannitol, 24.2% DMSO, 16.8% 1,2-propanediol, and 14% formamide

CP: 10% FCS, Penicillin-streptomycin, M199, and 2M DMSO

7C

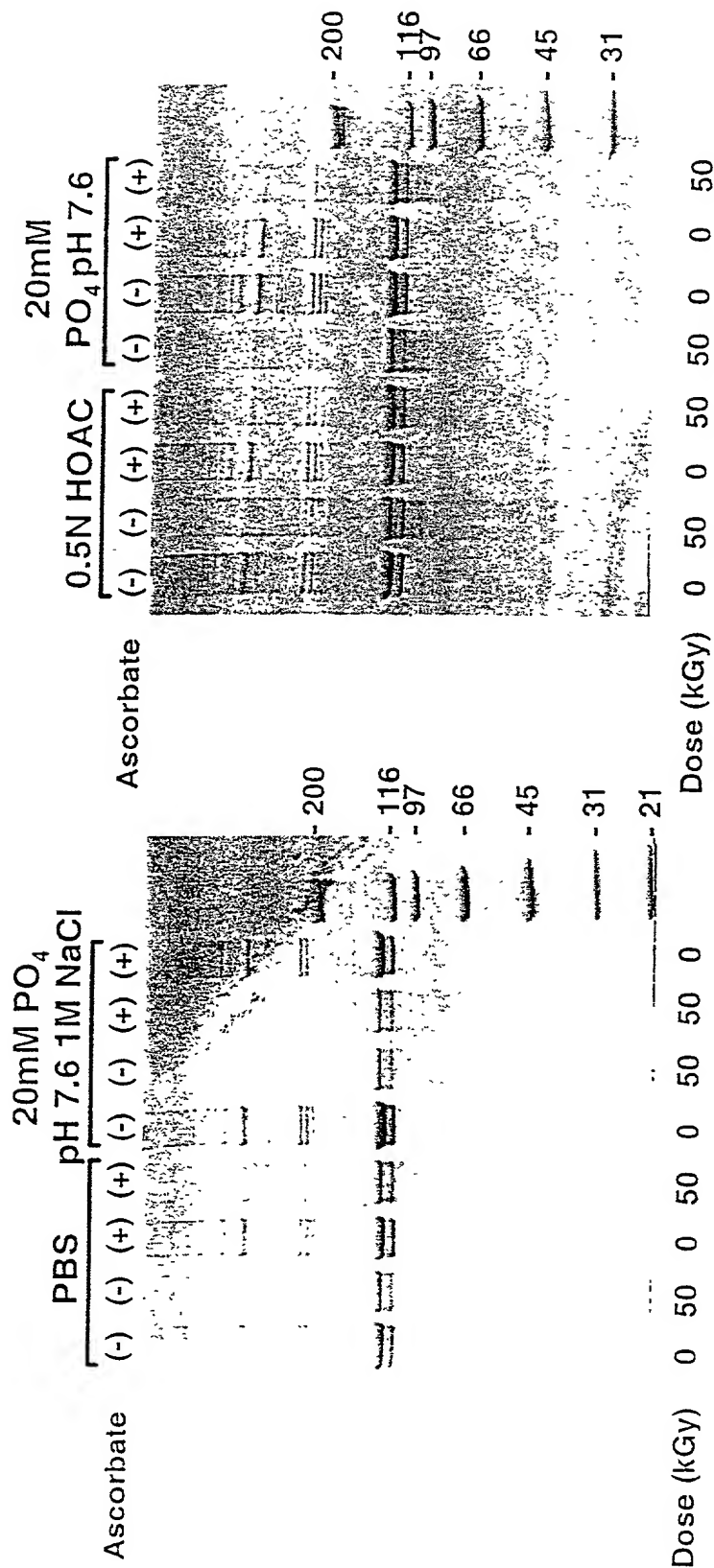
Pepsin-Digested Collagen Isolated from Irradiated Freeze-Dried Porcine ACL in the Presence of Antioxidants at 4°C 1.656kGy/hr



Cocktails: 100μM trolox, 100mM ascorbic acid, 100mM lipoic acid, 100mM n-propyl gallate

Fig. 8

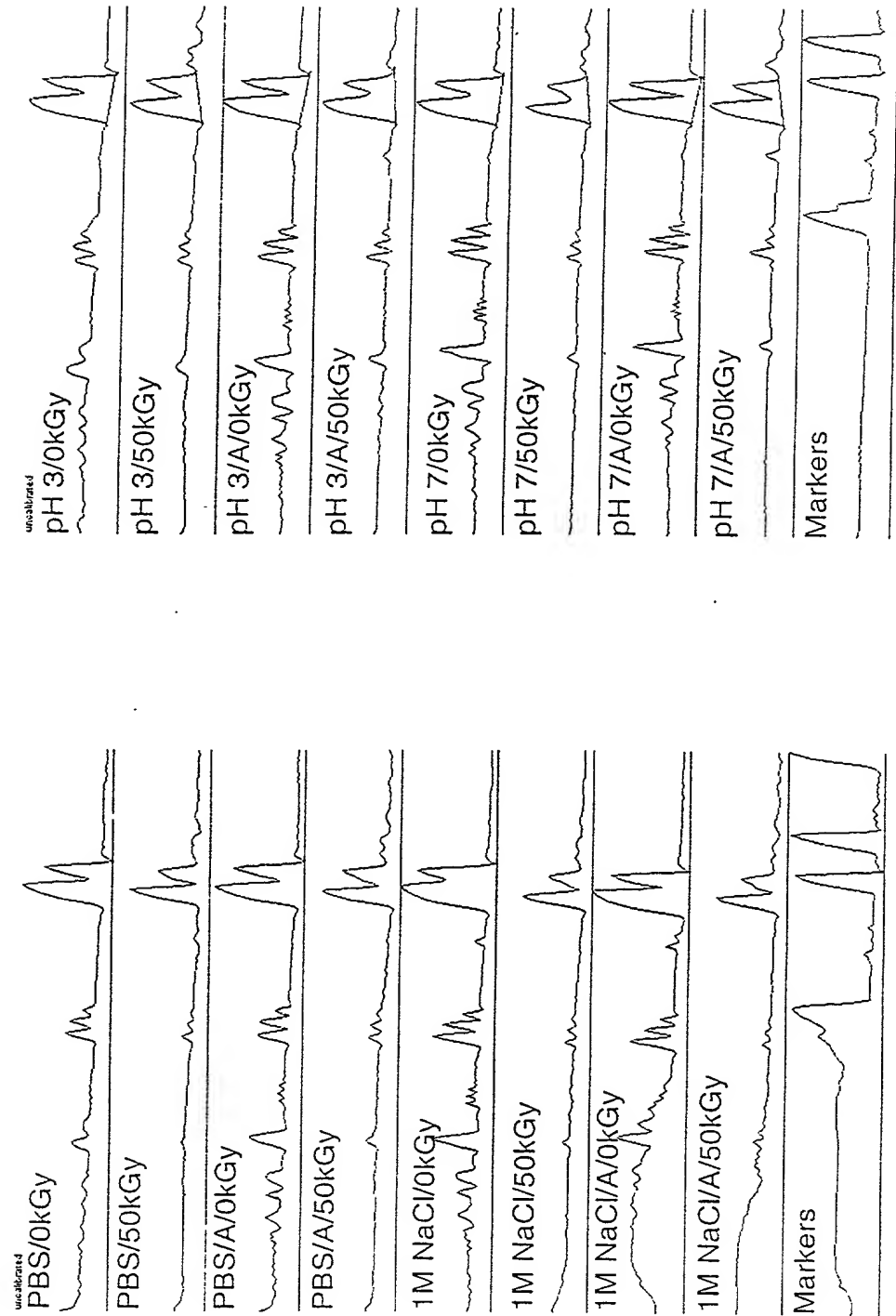
Pepsin-Digested Collagen Isolated from Irradiated Porcine ACL in the Presence of Ascorbate at -80°C 1.53kGy/hr)



GA

UNIVERSITY OF CALIFORNIA, SAN DIEGO
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PAGES 1-10

Densitometry of Pepsin-Digested Collagen Isolated from Irradiated Porcine ACL in the Presence of Ascorbate at -80°C 1.53kGy/hr



96

Pepsin-Digested Collagen Isolated from Irradiated Porcine ACL in the Presence of Scavengers at -80°C 1.53kGy/hr

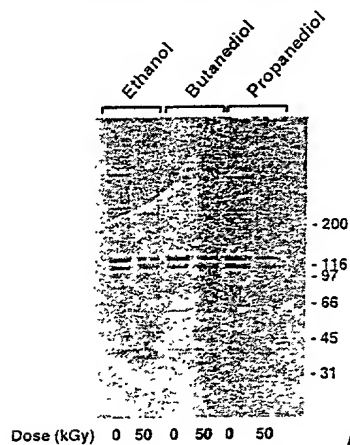
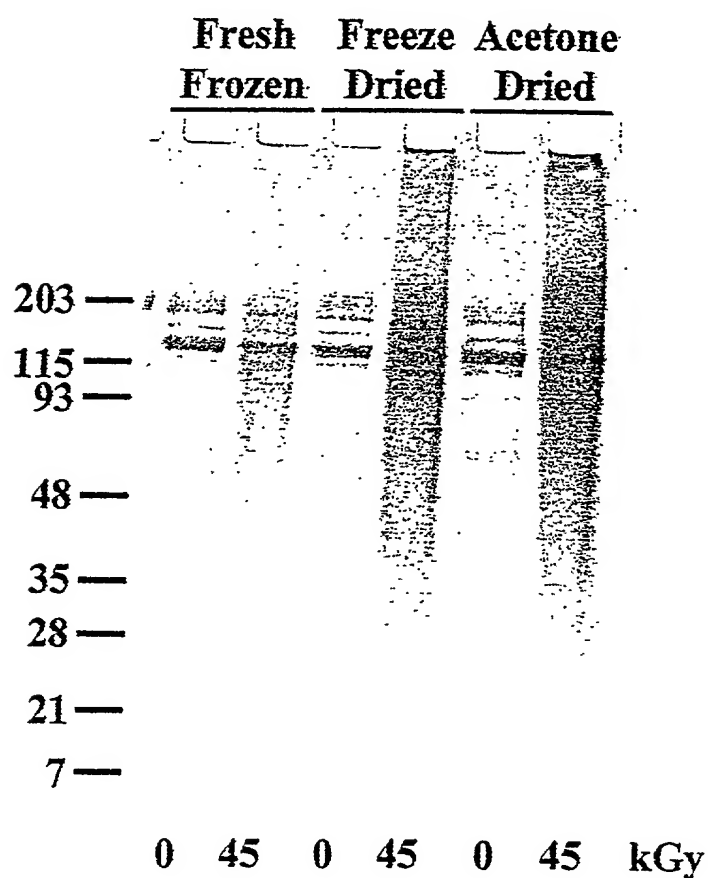


fig. 10

Low Temperature Gamma Irradiation of ACLs Subjected to Various Forms of Preservation -80°C, 1.5 kGy/h



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γ -irradiation of type I Freeze-Dried Collagen in the Presence of Antioxidants at 4°C 1.656kGy/hr I

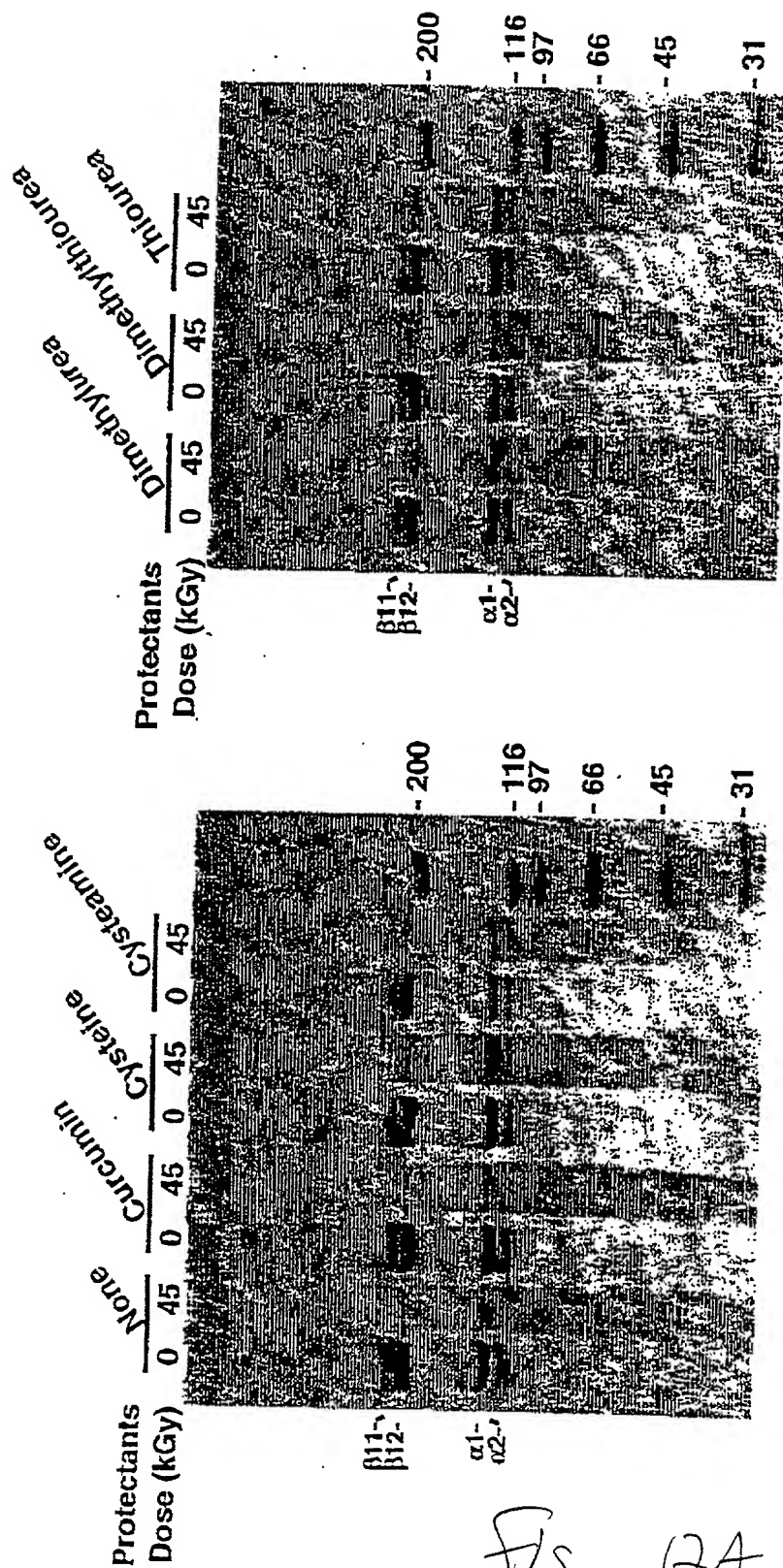


Fig. 12A

**γ -irradiation of type I Collagen Solution and Gel in the Presence of Antioxidants
at -20°C 1.537kGy/hr**

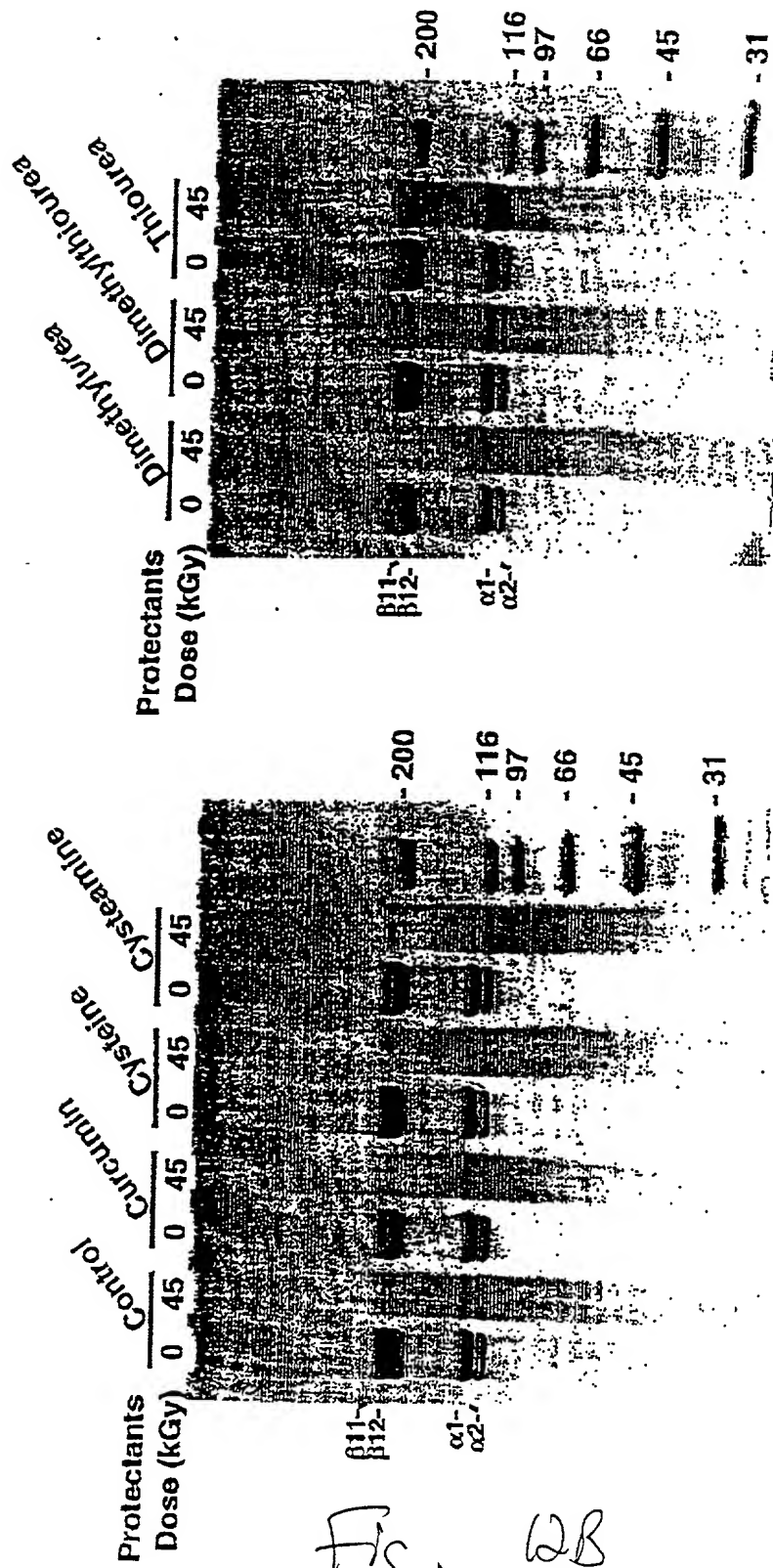


Fig. 2B

γ -irradiation of type I Collagen Solution In the Presence of Antioxidants at -80°C 1.53kGy/hr

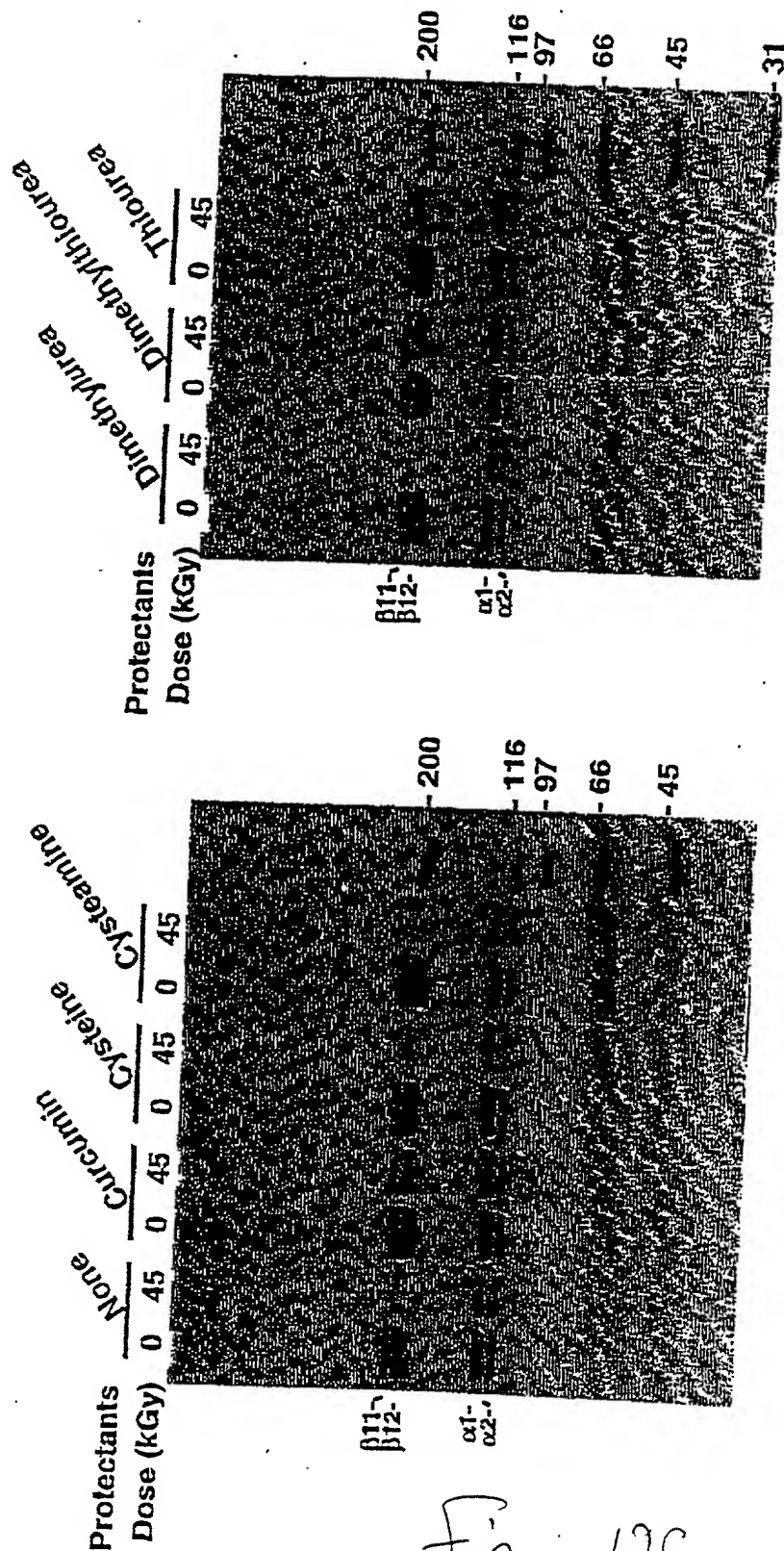


Fig. 12C

**γ -irradiation of type I Collagen Solution and Gel in the Presence of Antioxidants
at -80°C 1.3kGy/hr**

Protectants	Control			Asc			Asc/GG			Protectants			Asc/GG Methionine			Thiourea		
	Liq	Gel	Dose	Liq	Gel	Dose	Liq	Gel	Dose	Liq	Gel	Dose	Liq	Gel	Dose	Liq	Gel	Dose

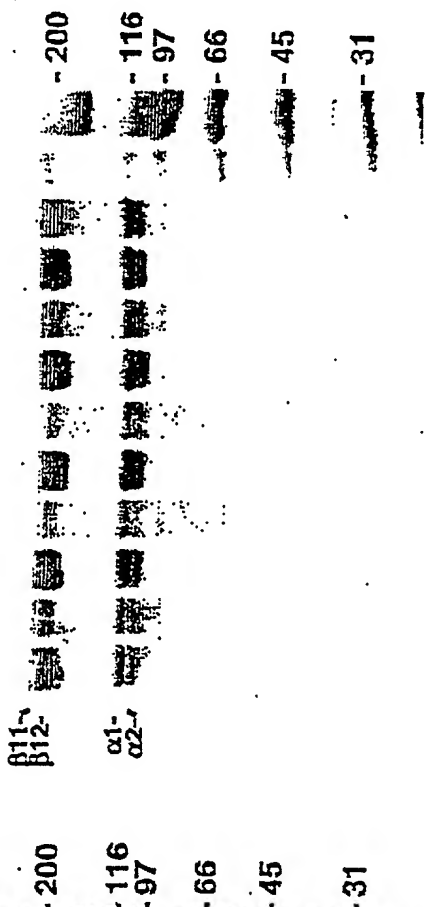
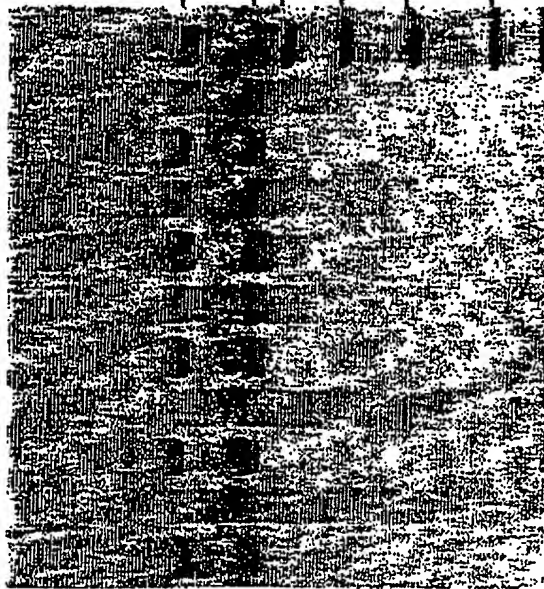


Fig. 13

γ -irradiation of Freeze-Dried type I Collagen in the Presence of Antioxidants at 4°C 1.673kGy/hr

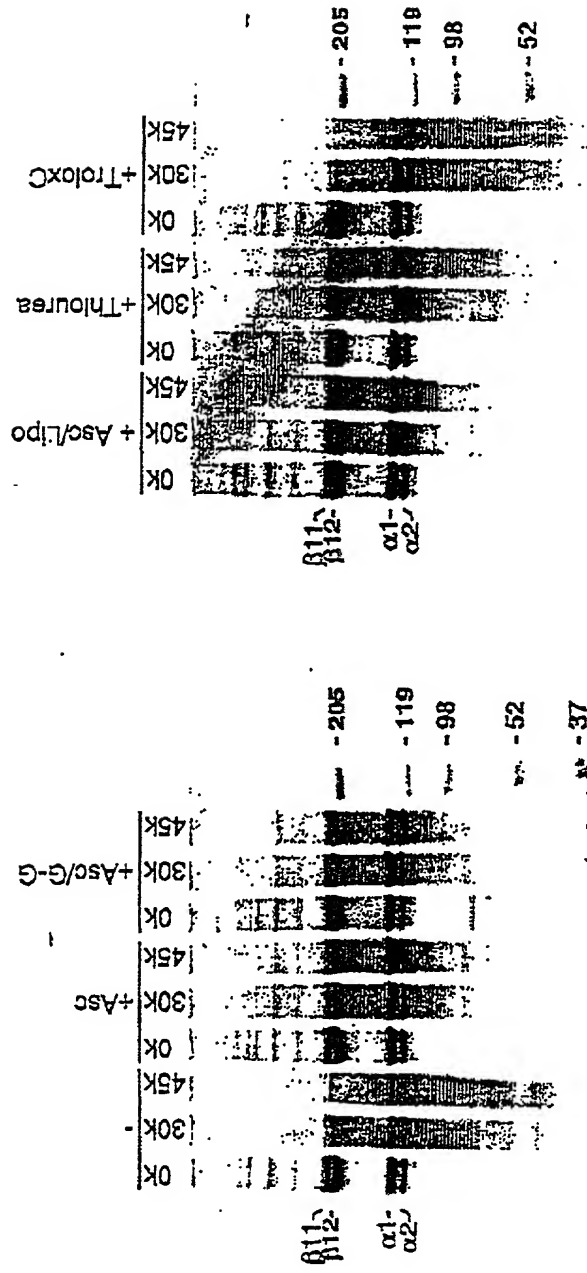


Fig. 14A

γ -irradiation of type I Collagen Solution in the Presence of Antioxidants at 4°C kGy/hr

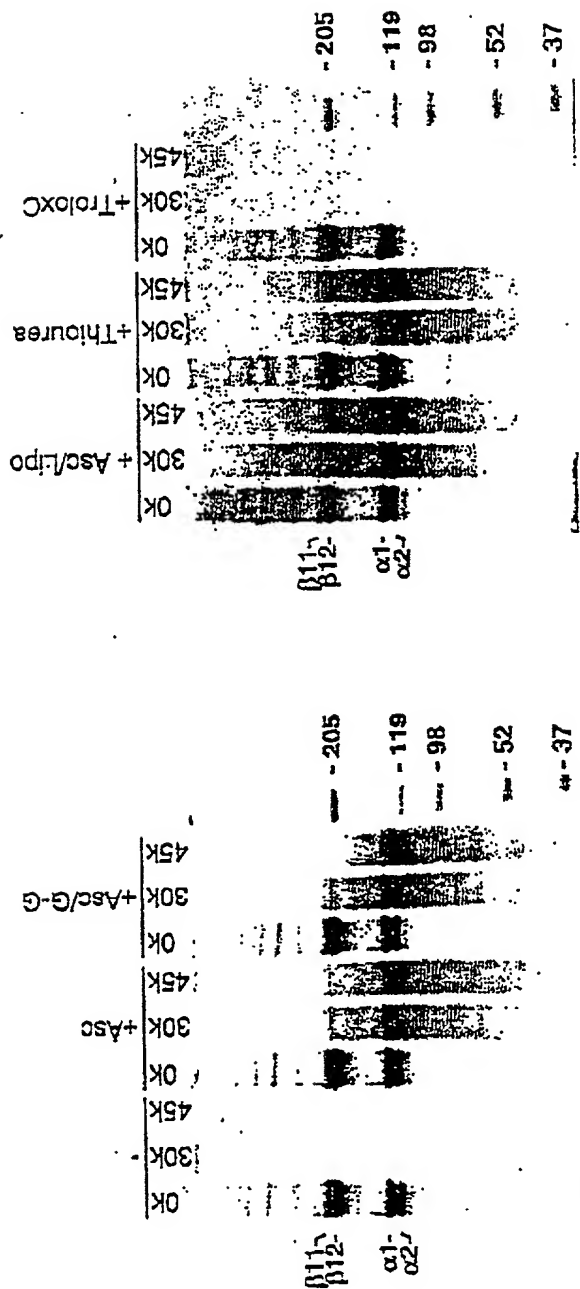


Fig. 14B

γ -irradiation of type I Collagen Solution in the Presence of Antioxidants at -20°C 1.552kGy/hr

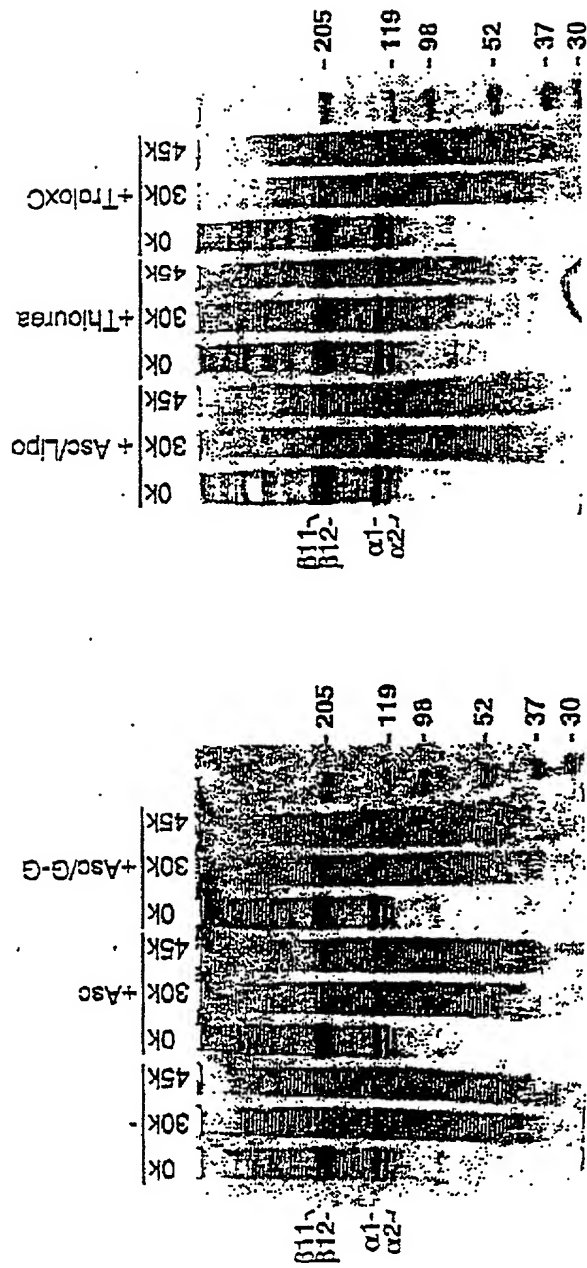


Fig. 14C

γ -irradiation of type I Collagen Solution in the Presence of Antioxidants at -80°C 5.136kGy/hr

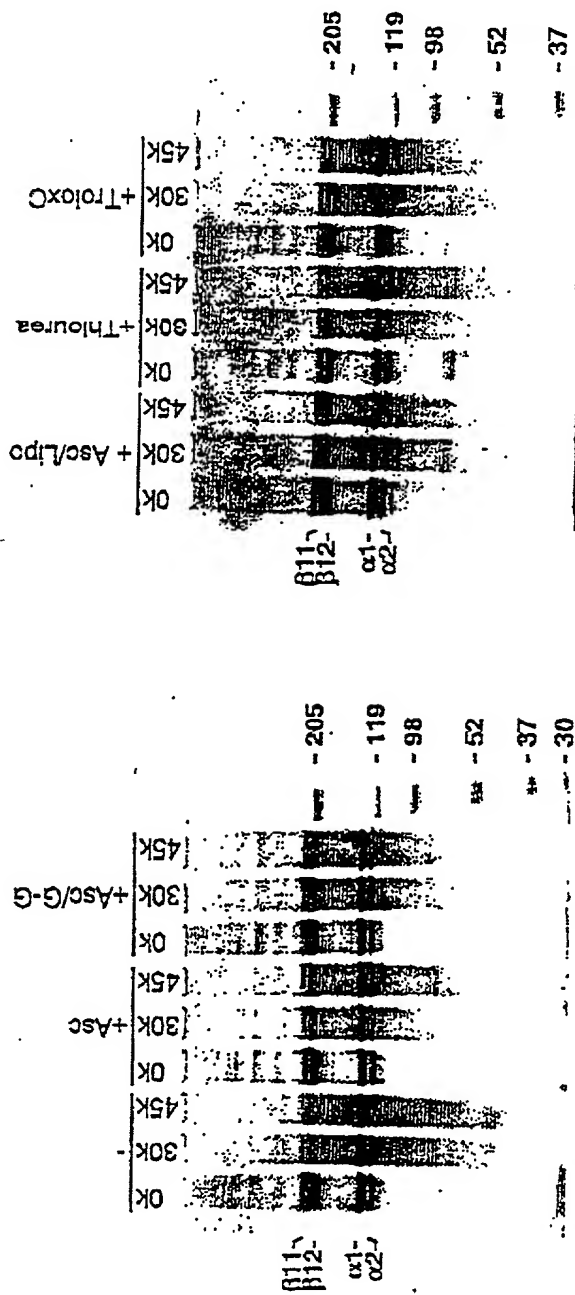


Figure 14D